PACHYDERM

NEWSLETTER OF THE AFRICAN ELEPHANT AND RHINO SPECIALIST GROUP

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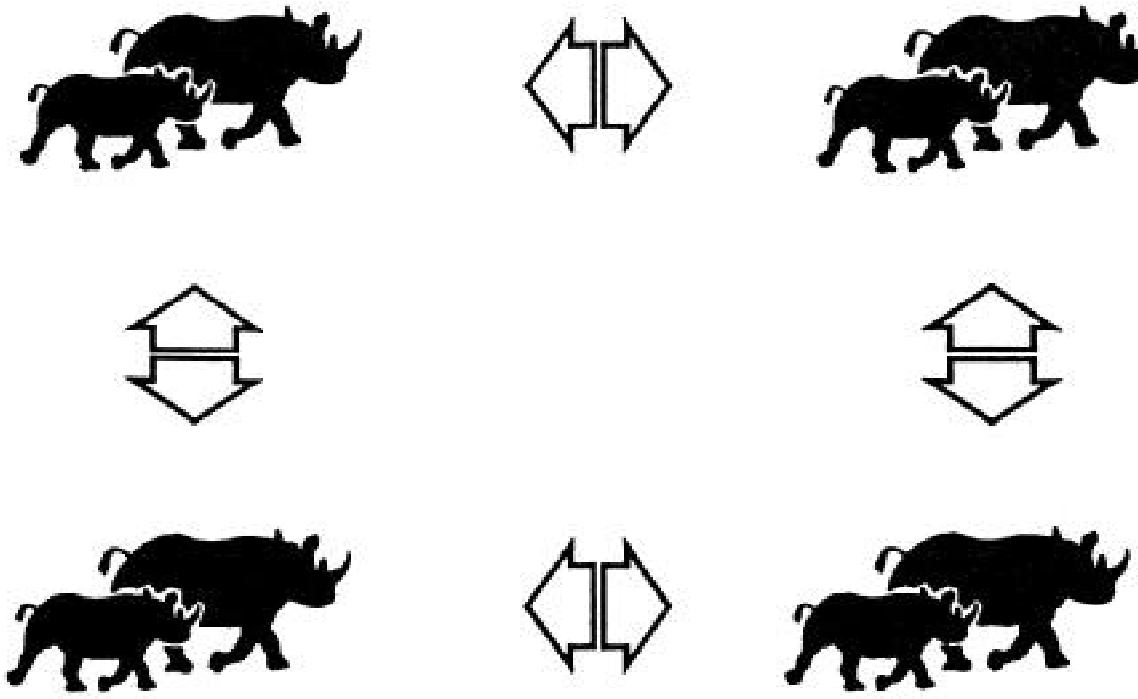

SPECIAL ISSUE

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Edited by R.F. du Toit, T.J. Foose and D.H.M. Cumming

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Proceedings of African Rhino Workshop

INTRODUCTION

Rhinos in Africa are in a crisis situation. Numbers of black rhinos in Africa have been reduced, largely by poaching, from an estimated 60 000 in 1970 to less than 4 000 today. Moreover, many of the remaining animals are distributed in small and fragmented populations whose survival may be endangered by genetic and demographic problems even if they can be protected from poachers. About 150 black rhinos are maintained in the zoos of the world. Almost all of these animals are derived from the East African populations.

The northern subspecies of the white rhino has declined to even tower numbers, with a maximum of only 20 animals known to survive in the wild In Africa. About a dozen animals are maintained in captivity, 9 of them (including all of the females) at a single institution, the zoo at Dvur Kralove, Czechoslovakia.

In response to this crisis, an African Rhino Workshop convened at the Cincinnati Zoo, 25.28 October 1986. The Workshop was organized by Cincinnati Zoo, the King's Island Wild Animal Habitat, and the AAZPA Conservation Coordinator's Office in consultation with the IUCN/SSC African Elephant and Rhino Specialist Group (AERSG) and Captive Breeding Specialist Group (CBSG). The Workshop was supported by a number of zoological organisations and institutions in North America including the AAZPA Conservation Endowment Fund.

Approximately 100 persons participated in the Workshop, representing field conservationists, zoo professionals, academic researchers, and support organizers from Africa, North America and Europe. The chairmen and many members of the AERSG and CBSG were in attendance. Lists of participants and sponsors are appended.

The Workshop was organized to persue several objectives:

(i) to contribute to development of the global strategy to conserve African rhinos;
(ii) to integrate and coordinate field and captive programs to preserve African rhinos, and especially to delineate how zoos can assist more with attempts to preserve these species;
(iii) to apply the principles of conservation biology, especially genetic-demographic management and decision analysis, to conservation of African rhinos.

The Workshop participants discussed problems and potentials for these objectives for three days. As a result, a number of recommendations and resolutions were adopted.

RECOMMENDATIONS

GENERAL

1. The Workshop emphasizes that continued poaching for the illegal trade in rhino horn is the greatest threat to the ultimate objective of survival of African rhinos in the wild, both as species and as components of their ecosystems. Therefore, the Workshop strongly encourages continued and intensified anti-poaching measures. Further, the Workshop urges continued and intensified efforts to reduce and eventually eliminate the trade in rhino horn, in particular, the Workshop urges the Organization of African Unity (OAU) and its member nations to apply pressure on those African countries harboring culprits to Implement all measures necessary to eliminate poaching and illegal trade in rhino horn and other products.

2. To facilitate the *ex situ* programs for African rhinos, the

Workshop observes the great need for annual updates of the international Studbook for both black and white rhinos. Moreover, the Workshop suggests that there be consideration of using studbook techniques for intensive *in situ* management of rhinos In Africa. The zoo community is able and willing to help African nations technically and financially with this endeavour.

3. The Workshop believes there is a need to improve the clinical and pathological investigations of both black and white rhinos in captivity and where practical in the wild. In this regard, the Workshop recommends that there be consideration of formulating and implementing standard methods of recording information collected in these investigations, It Is also desirable to preserve and Inventory biological samples, including osteological material.

4. The Workshop recommends that research be conducted on enhancement of reproduction in rhinos to provide techniques for transfer of germ plasm or genetic material which can be used for genetic and demographic management of captive and wild populations to assist In their survival. These techniques could reduce the costs and risks of moving live animals for management purposes and could permit more rapid expansion of under-represented genetic bloodlines of rhinos.

This research would Include oestrus detection and synchronization; the collection, analysis and cryopreservation of germ plasm; artificial insemination; and embryo transfer technology.

A researcher, working with an established reproductive research group, is needed to coordinate efforts currently underway and to conduct further specific projects. Such an effort will need to be funded for 3-5 years with direct costs of about $65 000 per year. The responsibility for organizing this effort has been accepted by the AAZPA SSP Species Coordinators for Black and White Rhinos.

5. The Workshop recognizes the usefulness of *Pachyderm,* the Newsletter of the AERSG, as a primary reference on rhino conservation, Issues and priorities. Therefore, the Workshop urges wider distribution of *Pachyderm,* especially to *ex situ* facilities and fund-raising organizations. Further, the Workshop endorses the idea of including in *Pachyderm* issues a status update with the most recent reports and estimates of numbers of rhinos in Africa. Further, it would be useful if AERSG regularly produced a list of the prioritized rhino projects, along with their costs, as an aid to fund-raising efforts and coordination.

BLACK RHINOS

1. The Workshop endorses the draft continental strategy for black rhinos in Africa formulated by the AERSG.

2. The Workshop reaffirms that the three major components of the conservation strategy for black rhinos consist of:

(i) protection of the larger (more than 100 animals) populations in the wild;
(ii) intensive *in situ* management of smaller (less than 100 animals) populations in the wild;
(iii) *ex situ* programs, specifically captive propagation, to reinforce survival of wild populations.

3. As an interim strategy, until more is known about the genetic and ecological differences within the species, the Workshop recommends that the intensive *in situ* and the *ex situ* programs recognize four conservation units within the black rhino range:

(I) the southwestern populations in Namibia;
(ii) the southern-central populations extending from Natal through Zimbabwe and Zambia into southern Tanzania;
(iii) the eastern populations in Kenya and northern Tanzania;
(iv) the north-western populations extending from the horn of Africa to Central African Republic and Cameroun.

Ex situ and Intensive *in situ* programs should not mix animals from these four conservation units at this time.

4. Appropriate studies of evolutionarily significant differences, including both genetic diversity and ecological adaptations, are greatly needed for management decisions concerning both the wild and the captive populations. The Workshop recommends that such studies be conducted as soon as possible and be coordinated through the AERSG. Hence the Workshop recommends that necessary funds be recruited for these studies. Specifically, the Workshop urges that $10 000 be sought by the zoo community, through the AAZPA SSP Species Coordinator, for the genetic studies of black rhino being conducted by Dr. Don Melnick in liaison with the AERSG. This $10 000 would represent matching funds for the $10 000 already offered by the New York Zoological Society to cover the estimated $20 000 total cost of this project.

The Workshop also encourages both field and zoo programs to provide sample materials, as requested and where practical, to Dr. Melnick for these studies.

5. For the long-term conservation of the species, the Workshop urges continuation and extension of the analysis of demographic and genetic problems for the species in the wild through population modelling and decision analysis. The appropriate experts on both captive and wild communities should collaborate on these studies; African governments are encouraged to cooperate with these initiatives.

6. Considering principles of conservation biology, the Workshop acknowledges that a minimum, long-term objective for each of the recognized conservation units in the wild is a total population whose genetically effective size Ne = 500. Since the genetically effective size is usually much lower than the census number, minimum populations for each conservation unit larger than 500 will be required to achieve this objective. If it can be assumed (based on comparison with some other species that have been studied) that Ne/N ratios in the wild will be the order of 0.25, a minimum total population of 2 000 per conservation unit may be required to achieve the objective of Ne = 500.

Since four conservation units are being recognized, these considerations suggest a minimum viable population of at least 8 000 rhinos in Africa as an optimal long-term objective. The Workshop also realizes that it is impossible that a contiguous population of 2 000 within any conservation unit will build up in the foreseeable future. However, by interactive management of the several disjunct populations that will likely characterize each conservation unit, the overall N and Ne objectives of some of these combined populations should be achieved.

The Workshop observes that the total estimated population of black rhinos in Africa is less than half the total recommended minimum number of 8 000 and that only the southern-central populations are near the 2 000 MVP recommended for each conservation unit. Finally, it must be emphasized that these recommendations are for minimum numbers. it Is highly desirable that populations larger than the minimum be maintained.

7. Since the conservation units being recognized will extend across political boundaries, there wilt be a need for regional cooperation within Africa for the optimal integrated and Interactive management of the populations.

8. Recognizing the value of captive propagation as a back. up to *in situ* conservation, the Workshop recommends that action should commence immediately to establish viable foundations in captivity of the three conservation units of black rhinos not presently represented well in zoos. Genetic analyses suggest that a foundation for the captive population of each conservation unit optimally be at least 20 rhinos from the wild that reproduce in captivity. Only the East African populations are represented by this number of founders in zoos. The southern-central populations are represented by four founders at most. There are no known representatives of the southwestern or the northern-western populations currently in zoos.

Although the most endangered of the conservation management units is the northern-western population, acquisition of founder animals for all three conservation units not well represented in captivity should be pursued immediately and in parallel.

It is recommended that any new founders should be incorporated in the captive populations under auspices of the AAZPA SSP or similar management programs and should be placed in facilities with a proven record in black rhino reproduction.

9. Considering the plight of the northern-western populations, the Workshop urges that a rapid survey be conducted of these populations to determine what intensive *in situ* or *ex situ* action is possible and appropriate. It is recommended that the Chairman of the AERSG coordinate recruitment of a person or persons to conduct such surveys within the next six months. It is further recommended that recruitment of the financial support for such a survey will be coordinated by the AAZPA SSP Species Coordinator.

10. The Workshop observes that it will be desirable for *ex situ* programs and technology to be applied to conservation of black rhinos both in the African countries where the species occurs as well as in the zoos elsewhere in the world. Therefore, the Workshop agrees that the zoo community outside Africa should provide as much assistance as possible to African nations in developing intensive *in situ* management technology and facilities.

NORTHERN WHITE RHINOS

1. The Workshop recognizes that there are two conservation management units of white rhinos: the described southern and the northern subspecies. At present, the southern white rhino seems secure in the wild. However, the situation for the northern white rhino is critical. Although genetic studies are still inconclusive about the differences between these two units, it is believed the northern population should be conserved because of:

(I) its probable ecological adaptations to the very different habitats it occupies compared to the southern populations;
(ii) its probable resistance to endemic diseases not present in the range of southern populations;
(iii) the possible genetic diversity the population contains for the species;

(iv) the resources that have already been expended on its conservation, and the interest and willingness of Zaire to conserve the species;
(v) the flagship nature of the species for conservation in this region of Africa.

2. The Workshop recommends Integration of the conservation programs for the wild and captive populations. Ultimately, these programs are expected to entail exchange of genetic material between the wild and captive populations. Fewer than 15 founder animals are known to exist for both the small wild and captive populations. These founders are evenly divided between the wild and captive populations. However, over the short term it is recommended that no animals be exchanged between the wild and captive populations; at this time it is recommended that every effort be exerted to expand the wild and captive populations as rapidly as possible from their small founder bases.

3. The Workshop endorses continued support for the *in situ* conservation programs in Garamba National Park. In particular, the Workshop believes that, in addition to the activity currently occurring, funds should be provided for a field biologist who can be deployed continuously in the Park with the rhinos. Further, the Workshop also strongly recommends that there be an intensive effort to train Zairois biologists to continue with these conservation programs into the future.

4. With respect to expansion of the captive population, the Workshop acknowledges and commends the considerable efforts of Dvur Kralove, in collaboration with the IUCN/SSC CBSG, to enhance the captive breeding program, as reflected in the report and recommendations by CBSG chairman Dr. U.S. Seal and CBSG member Dr. D. Jones, issued after their visit to Dvur Kralove in February 1986. Many of these recommendations have been implemented, including some reproductive examination of females, the movement of a lone male rhino from London to Dvur Kralove, the initiation of a facility enlargement at Dvur Kralove, and collection of samples for genetic analysis.

However, further analysis and evaluation of both the captive and wild population emphasizes the urgent need to expand the captive nucleus as soon as possible. Concerns over the demographic risks of maintaining the entire captive nucleus in one facility have intensified.

Therefore, the Workshop recommends that Dvur Kralove consider movement of 112 adult animals to another facility with experience in breeding the southern white rhino. Further, the Workshop recommends that Dvur Kralove be requested to suggest a timetable by which, if further reproduction does not occur there, other relocations will be undertaken. The reasons for these recommendations relate to enhancement of reproduction and reduction of demographic risks, as will be explained more fully in a white paper to be prepared over the next few months by Dr. Jones and Dr. Seal.

5. The Workshop encourages the use of the southern white rhino for development of reproductive technology to help the northern white rhino.

6. The Workshop also encourages continued investigation of the genetic and ecological differences between the northern and southern forms. With respect to the genetic studies, both field and zoo programs are encouraged to provide sample materials as requested and where practical to Dr. O. Ryder and colleagues.

AFRICAN RHINO SYSTEMATICS

Session Chairman RAOUL DU TOIT

RATIONALE FOR INVESTIGATIONS OF AFRICAN RHINO SYSTEMATICS

Comments by David Western (New York *Zoological Society)*

To ensure that efforts to conserve rhinos in the wild as well as in captivity are maintaining the existing genetic diversity of the species, it is necessary to establish the "evolutionarily significant units" within the different species. In the case of the northern white rhino, there has been much debate over whether this""subspecies" is sufficiently different from the southern white rhino to merit the expense and effort required to maintain the last remaining population in the Garamba National Park, Zaire. Funds allocated to conservation of these northern white rhinos might be better spent on initiatives to conserve black rhinos, which have dwindled from about 15 000 at the time when this issue was first debated to a present level of under 4 000. The importance of subspecies designations thus requires critical review in order to assign priorities for rhino conservation action in Africa, but conservation Initiatives need not be delayed while the necessary research is undertaken.

In debating the significance of genetic differences between allopatric groups of rhinos, it is necessary to consider not only the need to maintain the evolutionary potential of the species by preserving overall genetic diversity, but also the need to maintain genetic traits that constitute specific ecological adaptations, allowing some of the rhinos to thrive in habitats which may be unfavourable for other members of the species. Attitudinal zonation of habitats in East Africa may be one important factor influencing ecological adaptations of rhinos.

A further aspect to consider in strategies for conservation in Africa is the likelihood that the recognition of a certain group of a spectacular "flagship species" as being different to other groups of the same species elsewhere gives Impetus to national and International efforts to save those animals and their habitats — the effort to protect the mountain gorilla in Rwanda has been a case of this—"political" aspect of systematics.

THE EXISTING BASIS FOR SUBSPECIES CLASSIFICATION OF BLACK AND WHITE RHINOS

Summary of presentation by Raoul du Toit (IUCN African Elephant and Rhino Specialist Group)

The efforts of Hopwood (1939) and Zukowsky (1965) in revising black rhino systematics did not greatly Improve the classification since these authorities erected subspecies on the basis of very small numbers of representative skulls, and in some Instances the skulls representing their subspecies were those of immature animals (notably the subspecies *holmwoodi).* In view of these deficiencies, Groves (1967) produced a revision which identified 7 subspecies, but sam-

ple sizes were still very low (only 2 of these subspecies were based on measurements of more than 10 adult skulls). Groves' breakdown was as follows (with sample sizes indicated in brackets):

Diceros bicornis b/corn/s (5)	South Africa ——Cape area;
D.b. chobiensis (4)	Southern Angola, Chobe area;
D.b. minor (23)	South Africa to Kenya;
D.b. michaeli (22)	Kenya and Tanzania;
D.b. ladoensis (6)	Northern Kenya and Sudan;
D.b. Iongipes (4)	Central Africa;
D.b. brucii (10)	Somalia and Ethiopia.

Confusion was introduced since Groves did not indicate in this paper that he believed his subspecies *bicornis* to be extinct. This was only made clear in a paper he co-authored with Rookmaker in 1978. Here they stated that *bicornis* was a very large rhino that was exterminated in Namibia and the Cape in about 1850.

Several zoologists continued to refer to *bicornis* as one of the surviving species in southern Africa. Ansell (1978), in his Mammals of Zambia, excluded *bicornis* but had previously stated (1974) that some living rhinos of southern Africa were of this subspecies, and in his recent work Smithers (1983) apparently follows Ansell's original classification; he states that *bicornis* occurred widely in the subcontinent and now has a restricted distribution (presumably meaning this to be Zululand), while he thought *minor* may occur in northern Namibia/Angola (he does not clarify how this fits in with *chobiensis).*

Joubert (1970) compared some Namibian rhino skulls with a sample from Natal. He may not have checked that all skulls were of fully-grown animals, but found that all the Namibian skulls were significantly greater than those from Natal. However, he calculated that the differences between the populations were below the level conventionally accepted for subspecies differences (i.e. the ranges of dimensions had more than 10% overlap) and said all the skulls were of the *bicornis* subspecies.

Rookmaker and Groves (1978) commented that *bicornis* (as described by them from Cape specimens) was similar to *chobiensis* in that both had large skulls, and postulated that this was due to independent adaption to similar (wet) environments. This is clearly fallacious, since the climates of southern Angola/Chobe and the Cape/Namibia are dissimilar, and are not wet.

Thus, the published literature contains rather confusing statements on black rhino taxonomy, and sample sizes are small. Dr. C. Groves recently sent the African Elephant and Rhino Specialist Group (AERSG) an outline of his current ideas on the topic, including data from a few more skulls. His new, interim classification is similar to that he published in 1967, but excludes *bicornis* as an extant subspecies, and has the following criteria for the taxonomic divisions: presence or absence of crista (a tooth feature), greatest length of skull, zygomatic breadth, toothrow length and occipital breadth. Three of the subspecies still have less than 10 representative skulls *(chobiensis, ladoensis* and *iongipes).*

In view of the poor state of black rhino systematics, AERSG Initiated a survey of black rhino skulls in African wildlife areas and in some museums. This survey is not complete, but initial results can be presented. The data indicate that there is statistically significant variation between certain dimensions of female skulls and the equivalent dimensions of male skulls from the same population (notably in toothrow, basilar length and zygomatic breadth). Groves' latest classification is not supported by the data; for instance, all the skulls that were measured In Etosha National Park have occipital breadths greater than the maximum range indicated by Groves (which was for *chobiensis).* The range in toothrow length which Groves gives for *brucil* totally covers the range he gives for *minor* and thus would be a poor distinguishing feature anyway), but there are a number of fully-grown skulls measured recently from supposed *minor* populations which have even shorter toothrow lengths.

The 300 skulls measured so far in the AERSG survey are mainly from southern Africa and thus only a very tentative conclusion can be reached on the clinal variation in black rhinos. This conclusion is that there may be possibly a trend of decreasing skull size towards the north of the continent, with the largest skulls being from the Namibia animals, a range of Intermediate sized skulls extending up to Kenya and possibly west from there to the Central African Republic, and small skulls from the population to the horn of Africa (Somalia and Ethiopia; where in fact the animals may be effectively exterminated by now). If there is a large-skulled rhino group in Namibia, this may well have been linked with the supposed *bicornis* population as well as with the *chobiensis* population; based on collection localities of skulls designated as *bicornis,* and on ecological similarities between the postulated range of *bicornis,* and that of the extant Namibian rhino, Hail-Martin (1985) has also suggested that these may be the same race.

Thus, in general, it would appear that taxonomic distinctions between black rhinos have been exaggerated and a concerted effort to measure more skulls is justified (the AERSG survey will now build up data from East Africa, but it is expected that few data will be forthcoming from Central Africa). The working premise of AERSG that efforts to conserve rhinos and to create captive breeding groups should concentrate on rhinos from either end of their current range in Africa and from the middle of the distribution is supported. It is also clearly important to undertake further investigations of the ecological adaptions (physiological and behavioural) which suit rhinos to particular environments (notably the Namibian desert and Kenyan highlands) ——adaptions to blood parasites may be particularly important, and would not be revealed by the classical taxonomic approach of measuring skulls.

There has been consensus between taxonomists in the identification of the two subspecies of white rhinos:—*Ceratotherium simum cottoni* and *C.s. simum.* However, these subspecies have been nominated largely on the basis of geographical separation ——several taxonomists have noted that on the basis of skull characteristics the two are not well differentiated. Groves (1972;1975) feels that the major difference is that *simum* has a much deeper dorsal concavity (the occipital crest is raised higher). There is an overlap of only 5% in the ranges of this dimension for the two groups thus the difference, taken in isolation, could be said to constitute a valid subspecies distinction (but, as with the black rhinos, the sample sizes were small ——less than 1 0 *simum* skulls were measured). On the basis of the less indented skull of *cottoni,* Groves (1975) postulates that this subspecies has evolved further than *simum;* he believes that the fossil record indicates an advance from *Diceros* via *C. praecox* to *C. simum* with the dorsal outline of the skull becoming flatter.

The other major skull difference between the subspecies is in toothrow length, with *s/mum* having a longer toothrow, but the coefficient of difference is too small for taxonomic separation on this character (there is an overlap in the ranges of

20%). Alexander and Player (1965) have also stated that the southern race, *simum,* has sparse body hair white the northern has no hairs, only follicles. Groves (1975) suggests that the northern may be longer-legged and shorter-bodied than the southern, but this is not based on any data.

A BRIEF PALAEONTOLOGICAL HISTORY AND COMPARATIVE ANATOMICAL STUDY OF THE RECENT RHINOS OF AFRICA

Summary of presentation by Claude Guerin
(Universite Claude Bernard ——Lyon)

Information on this subject has been published by Guerin (1980).

The black rhino (*Diceros bicornis)*

The lineage begins in the upper part of the middle Miocene, about 12 million years ago, with
Paradiceros mukirii known from Fort Ternan (Kenya) and Beni Mellal (Morocco). The genus *Diceros* appears later in the upper Miocene and is known at that time in Spain, Greece and Turkey with *D. pachygnathus,* In Turkey with *D. neumayri,* and in Tunisia and Italy with *D. douariensis.* The first of these three very large Miocene species may be the ancestor of the white rhino, *Ceratotherium.*

The species *D. bicornis* appears during the Pliocene about 4 to 5 million years ago, and is known in more than 20 sites of Pliocene up to middle Pleistocene age, especially Hadar (Afar) in Ethiopia, Omo (Mursi, Usno and Shungura formations) in Ethiopia, East Turkana in Kenya, Laetolil and Olduvai In TanzanIa. More sites of upper Pleistocene and Holocene age are recorded. However, the material is always rare and the fossil form has not yet received any precise taxonomic status. Anatomical differences between the fossil and extant forms are minimal. Thus the fossil form warrants no more than a subspecific status.

I have studied about 60 adult skulls and more than 30 postcranial skeletons of *D. bicornis,* most of these being of Groves' (1967) medium-sized East African forms: subspecies *ladoensis, michaeli* and *brucii.* It is not easy to distinguish between these subspecies, whereas *minor* appears to be smaller-skulled and *bicornis* exceptionally large-skulled. I have not been able to study *chobiensis* and *longipes.* Statistical analyses show that, from the data I collected, *D. bicornis* is homogeneous, with rather normal variability (see Guerin, 1980). The various subspecies appear to constitute a complicated cline.

The white rhino *(Ceratotherium simum)*

The lineage of the white rhino Is much more recent than that of the black. The genus

Ceratotherium appears during the Pliocene with *C. praecox,* a species defined in 1972 by Hooijer and Patterson with material from Kanopol and Ekora in East Africa. The same year Hooijer described abundant material of the same species from Langebaanweg In South Africa. I have studied the material from Chemeron formation (Lake Baringo) and a good deal of material from Hadar (Ethiopia) and from Laetolil (Tanzania). The species is now known in 11 localities of East and South Africa.

The recent species *C. simum* appears about 3 million years ago. it is classically held that there are two fossil subspecies, C.s. *germanoafricanum* from East Africa and *C.s. mauritanicum* from North Africa. I have studied material of *germanoafricanum* from Afar, East Turkana, Olduvai, Omo, Rawi and sever minor locations, and *mauritanicum* material from Ternifine (0.8 million years), Ain Hanech (1.5 mIllion years) and other minor localities. The postcranial material shows clear differences between the fossil and the recent subspecies.

For the two recent forms, *simum* and *cottoni,* I have been able to find only about 30 skulls and 12 postcranials, and many were without specified origin. In fact, only 16 skulls and 8 postcranial skeletons were certainly from *cottoni,* and 8 skulls with 2 postcranial skeletons from *simum.* Hence the results are little more than an indication of differences. On average, *simum* has a skull slightly larger than that of cottoni, with a lower and broader skull roof, and a differently-shaped occipital surface (confirming observations of Groves, 1975). Comparison of fossil forms with the complete sample of recent species shows that the skull of *C. praecox* Is shorter, broader and lower, while the skull of *C.s. germanoafricanum* seems like that of a gigantic white rhino with comparatively narrower occipital surfaces, broader cheek teeth and correspondingly narrower palate widths. A comparison of limb elements again shows *germanofricanum to* be like a giant white rhino, while *mauritanicum* has similar (or exaggerated) proportions to *C. praecox,* being dissimilar to recent white rhinos and *germanoafricanum.*

Since the two Pleistocene subspecies seem to be very different to each other and from the recent ones, *germanoafricanum* probably deserves full species rank and may be the ancestor of the two recent forms; *mauritanicum,* which has no descendants, seems closer to their common ancestor, *C. praecox,* and probably also deserves species rank. The two recent subspecies are clearly distinct from each other and seem to be In the course of a speciation process. More postcranial material, particularly from southern Africa, Is required to help verify this.

BIOCHEMICAL INVESTIGATIONS OF RHINO SYSTEMATICS

Summary of presentation by Matthew George
(Howard University)

A comparative study was undertaken of genetic differences between individual northern and southern white rhinos, and a black rhino. This study was based on comparisons of mitochondrial DNA (mtDNA), which is a useful means of Investigating closely related species since 1.) the molecule Is maternally Inherited, thus complications arising from paternal contributions and recombination events (which affect nuclear DNA) are avoided; 2.) the molecule evolves very rapidly (5-10 times faster than nuclear DNA) so that if differences exist between races they are more likely to be detected than through other methods.

After purification of mtDNA molecules extracted from liver and spleen tissue of the three animals, these were subjected to digestion by 21 different restriction enzymes (which cut the mtDNA at specific sequences of nucleotide units). The cleaved fragments were separated electrophoretically. With most of the restriction enzymes, the migration patterns of mtDNA of the black rhino were different to those of the two white rhinos, while comparison of the two white rhinos showed 13 patterns to be identical and the remaining 8 different.

Analysis of these data indicate that the white rhinos differ by 4% In their nucleotide sequence and they both differ by 7% from the black rhino, If rhinoceros mtDNA changes at a rate of 2% per million years as has been shown in primate mtDNA, the divergence time between the white rhinos is 2 million years, and between either of the white rhinos and the black

rhino is 3.5 million years. The estimated time of divergence between the two species agrees well with fossil evidence (Hooijer, 1969), but the two million year divergence time for the two geographically separated subspecies is surprising; the mtDNA analysis suggests that little or no gene flow has occurred between the races for this period.

The intraspecific variation in mtDNA observed here in the white rhino is consistent with levels of intraspecific variation found in other species such as macaques, apes, rodents, sheep and goats. The intergeneric difference (7%) for the mtDNA of *Ceratotherium* and *Diceros* is somewhat lower than observed in mtDNA studies on other taxa.

We may tentatively conclude that, whereas morphological divergence between *simum* and *cottoni* has been slow (due perhaps to similar selection pressures or convergent evolution), the mtDNA analysis exposes significant genetic differences in these two forms. A second *C.s. simum* individual's mtDNA was subsequently studied, with essentially similar results. However, more sampling is required, in particular to verify the basic level of intraspecific variation in a particular race of white rhino, so that we can be certain that the differences between the northern and southern races are not in fact normal intraspecific polymorphic differences. in addition to increasing the sample size (ideally about 10 rhino from each race should be studied), the number of restriction enzymes could also be increased. *Comments by Oliver Ryder (Zoological Society of San Diego)* While the analysis of mitchondrial DNA of northern and southern white rhinos displays clear differences, no significant differences have been elucidated from protein electrophoretic studies carried out at the University of California, San Diego (A. Merenlender and D._Woodruff). Twenty-six presumptive loci were examined from five northern white rhinos, 14 southern white rhinos and five black rhinos (all *michaeli).* The electrophoretic difference between the northern and southern forms was approximately one-tenth that between white and black rhinos, whereas the. mitochondrial DNA studies had shown a difference between the northern and southern races which was about one-half of the mitochondrial DNA differences between the white and black rhinos.

Additional samples of northern white rhinos have been obtained from animals in captivity at Dvur Kralove, Czechoslovakia and will be subjected to mitochondrial DNA analysis. Additionally, chromosome studies of both black and white rhinos are very limited and should be undertaken. Both of these projects are underway in research supported by the Zoological Society of San Diego and the Ellen B. Scripps Foundation.

The phylogenies derived from fossil, electrophoretic, and mitochondrial DNA studies agree, but questions arise over the rates of evolution and times of divergence between the taxa. It is known that the rates of divergence in different animal lineages vary greatly and it would seem that the genetic loci studied by protein electrophoresis may have a particularly slow rate of evolution in rhinos in comparison to other vertebrates. This is consistent with the mitochondrial DNA findings. The fact that the protein electrophoretic studies indicate that genetic distances between the northern and southern white rhino are no more than those that can be expected in a single randomly mating population, while the mitochondrial DNA studies indicate longstanding genetic isolation, may be due to the difference in rates of evolution of nuclear genes (assayed by protein electrophoresis) a mitochondrial DNA or they may be due to a rehybridization event. Limited breeding occurring between rejoin populations that had been separated for some time has led merging of nuclear genes with retention of mitochondial DNAs of only a single population. Generally, the phenomena require recent genetic interaction of the previously separated populations.

While conservation decisions may need to be ma immediately, a clearer understanding of the systematics both white and black rhinos will require further studies chromosomes, protein electrophoresis, mitochondrIal a nuclear DNA genes.

Comments by Don Melnick (Columbia University)
In applying genetic methods to conservation goals we must be careful to avoid placing too much importance on subspecies designations and, instead, assess the distribution of genetic, morphological and ecologic variation throughout a species' range. It is these variants that we wish to conserve in the most efficient, cost-effective w and not the somewhat arbitrary taxonomic distinction between so-called subspecies.

With this in mind, it is necessary to investigate the distribution of genetic diversity (Nel, 1973) across the remaining black rhinoceros populations, in order to establish how much of the species variability can be attributed differences within populations as opposed to difference between populations. This will help us avoid some of the difficulties which have arisen in interpreting the results oft white rhino studies.

The relevance of genetic diversity analysis to rhino conservation in Africa can be Illustrated by an example of two Asian primate species (Melnick, 1987). Only 5% of the genetic diversity found among rhesus monkeys across Asia can be attributed to differences between animals in different regions. The remaining 95% of species diversity is 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 difference between regional populations of this species. Hence, if the strategy were devised to conserve the greatest amount on genetic diversity in these primates it would entail the conservation of many more regional populations of the long-tailed macaque than the rhesus monkey. Given the scarcity of resources available for the conservation of the black rhino, we need to determine which of these two types of genetic structure exist.

With the assistance of the New York Zoological Society, the AAZPA and the AERSG, a genetic survey of the black rhino has commenced, with the aim of analyzing mtDNA and bloc proteins in reasonably-sized samples from populations different parts of Africa. Thus far, blood samples from 3 individual black rhinos have been collected in Zimbabwe by P. du Tout, sampling is underway in Kenya and some samples may also become available from South Africa. Sampling very opportunistic, since it usually depends on translocation exercises. It may be very difficult if not impossible to get samples from central Africa. In addition to the wild-caught rhinos, we have collected, with the help of participating zoo blood samples from 12 captive rhinos of Kenyan origin. protocol for tissue collection has been developed and ha been circulated to those who may be in a position to obtain samples.

ECOLOGICAL ADAPTIONS OF RHINOS

Summary of discussion

N. Owen-Smith noted that the feeding ecology of northern white rhinos may well differ to that of the southern white rhinos. The latter graze on short, nutritious grasses while the northern animals live in a wetter habitat, with long fibrous grasses. K. Hillman-Smith confirmed that this is a possibility but relevant research has not yet been undertaken in Garamba National Park. Casual observations indicate that

the northern rhinos may eat more dicotyledons than the southern, and they have to survive in tall grasses he such as *Hyparrhenia* and *Loudetia* in the wet season, and in burnt areas during the dry season. Their social behaviour appears similar to that of the southern rhinos although ranges are about 10 times larger; this may be due to the very low population density in Garamba.

It was generally agreed that estimations of divergence times, subspecies designations and other phylogenetic/taxonomic aspects do not necessarily allow us to identify "evolutionary significant units" (ESU's). Important ecological adaptations may remain hidden from biochemists investigating genetic material and blood proteins, and will almost certainly not be picked up through skull measurements, so it is necessary to investigate the range of habitats in Africa (with their varying selection pressures) in order to outline common-sense strategies for both continental and national rhino conservation initiatives. If a group of rhinos from one part of the species' range is not likely to adapt to different environmental factors when moved to another part of the range, then it is obviously important to conserve representatives of the original populations of both regions.

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APPLICATION OF DECISION ANALYSIS TO BLACK RHINOS

Discussion Leader LYNN MAGUIRE

INTRODUCTION

Purpose

The presentation had three purposes: (if) to introduce several issues crucial to the management of small wild or captive populations; (ii) to propose for discussion some strategies for the coordinated management of wild and captive populations of black and white rhinos; and (ill) to examine two elements of the proposed strategies using formal methods for decision making under uncertainty. These methods have proved useful in developing management plans for other endangered species, including black-footed ferrets (Maguire, 1987a) and tigers (Maguire, 1987b).

Small population management

Several features of the demography and genetics of small populations have important implications for their management.

(I) The concept of *minimum viable population size* (MVP) (Schaffer, 1981) suggests that populations cannot be self-sustaining below some minimum level. Small populations are particularly vulnerable to extinction due to stochastic fluctuations: demographic (e.g. sex ratios at birth), environmental (e.g. variations in food supply), catastrophic (e.g. fire), and genetic (e.g. fixation of deleterious alleles).

(ii) Due to nonrandom mating systems, unequal family sizes, fluctuating population size, and other factors, real populations have an *effective population size* (Ne) that is often far lower than census size, which means that genetic variation Is lost much faster than would appear on the basis of total numbers. Loss of genetic variation is a concern because variation is the raw material for short and long term fitness, in the wild and in captivity.

(iii) Although a relatively small number of founders can capture most of the variation from a larger population initially, this variation will be lost quickly if the population stays small. Black rhinos have declined quickly, suggesting that the remaining animals may provide a good sample of previous levels of genetic variation, but not for long.

(iv) In long-lived species, such as rhinos, numbers may give a misleading impression of population status, because numbers may remain stable while reproductive rates and age structure show a population in serious difficulty.

Assumptions

Several assumptions about rhino taxonomic and population status coloured the decision analysis. I assumed that the designated subspecies of black rhinos (Groves, 1967) are part of a continuum of geographic variation over the species range, and that the two white rhino subspecies are likely more divergent than black rhino subspecies. The subspecies populations of black and white rhinos can be divided into two groups: those in immediate danger of extinction *(D.b. ladoensis, longipes, chobiensis, brucii,* and *C.s. cottoni)* and those with somewhat larger and/or more stable populations *(D.b. bicornis, michaeli, minor,* and *C.s. simum).* Either many of the sociopolitical factors contributing to the rapid decline of African rhinos will change in the next few years, or rhinos will become extinct: therefore, a very short time frame for rhino management decisions, say 15 years, seems appropriate. This is less than one rhino generation: genetic effects will not be evident within that period, but managers must be careful not to set up situations that may be successful in the short run, but disastrous in the longer term.

INTEGRATED STRATEGIES FOR WILD AND CAPTIVE MANAGEMENT

Objectives

Some of the possible objectives for rhino conservation include: (if) maximizing survival probability at the species, subspecies, or population levels; in the wild population, the captive population, or overall; (ii) maximizing retention of genetic variation for any of these categories; (iii) maximizing the number of subspecies or geographic populations surviving; (iv) maintaining the geographic range and habitat of rhinos; and (v) minimizing financial costs. Some of these goals may conflict.

Management strategies

The options for managing wild and captive rhinos may be organized into a hierarchy, ranging from least to most intensive: (ion) control of poaching and protection of habitat for wild rhinos; (ii) intensive management of wild populations. including translocation of animals among isolated populations; (ii) semicaptive (intensive *in situ)* management of rhinos in heavily protected, often fenced, areas; and (iv) captive management in zoos *(ex situ* management). Ideally, all levels of management should be coordinated, allowing exchange of animals as needed to maximize effective population size of each management unit. To date, most movements have been from the less to the more intensively managed situations. The challenge for rhino conservation is to develop a coordinated management plan using all levels in the hierarchy.

Because the focus of this workshop was the use of captive management in rhino conservation, the decision analyses presented here deal with the allocation of zoo space to the geographic units of African rhinos. The special strengths of zoos are: (I) reducing mortality, including human predation; (ii) enhancing reproduction through intensive management of captive breeding; and (iii) maximizing retention of genetic variability through intensive genetic and demographic management of captive animals.

For the near future, about 400 to 450 spaces are available for African rhinos in zoos which can be considered part of an SSP program. At present, of the seven extant black rhino subspecies, only *D.b. michaeli* (143 animals) and *D.b. minor* (5 animals) are represented in the captive populations. Only 11 *C.s. cottoni,* but 370 *C.s. simum,* represent the two white rhino subspecies. Given the relative status of the subspecies In the wild, this allocation of captive space is far from optional. Given also that a population size of around 100 animals per Interbreeding unit is desirable for long term captive management (to retain genetic variability), and that there are only about 450 spaces for nine African rhino subspecies, allocation of available space is difficult.

In weighing the reallocation of captive space among subspecies, several considerations arise. Because there is not enough captive space to maintain viable populations of each of the nine subspecies, it will be necessary either to exclude some subspecies from the captive program or to mix some subspecies in a single management unit.

Considerations in mixing subspecies, or even geographic populations, include the potential for outbreeding depression and the loss of genetic and nongenetic adaptations to particular environments. For example, *D.b. bicornis* in the southwestern deserts exhibits behavioural adaptations to the harsh climate. Availability of founders is a concern for the severely endangered black subspecies, none of which are currently represented in captive populations, It is generally undesirable to initiate a captive program with fewer than 15 to 20 founding animals (not necessarily obtained simultaneously). It may be impossible to obtain such a founding population for any of the moat endangered black subspecies: indeed, it may be difficult to obtain 20 founders for a mixed population of these subspecies, even at great expense. Maintaining fewer than about 100 animals in a captive management unit may be justified if continued opportunity for exchange of animals, or genetic material, with wild populations is likely. Therefore, for those subspecies with more stable wild populations, such as *C.s. simum,* a smaller captive program may be desirable. In any case, maximizing the productivity and the retention of genetic variability from all captive rhino populations is a priority, toward which redistribution of current captive animals and research on better captive management of reproduction and mortality should be aimed.

To make the captive program better serve the needs of rhino conservation, substantial reallocation of space will be required over a period of years. The plan should be adaptive, responding to changes in both wild and captive status during the transition period. The important points for the near term are to understand what initial steps are required, and what events in the wild should dictate a change in captive strategy.

CAPTIVE PRIORITIES

Capture of additional wild rhinos seems unavoidable if the captive program is to serve as a repository of genetic material and a source of animals in the future. in view of the current holdings of subspecies in captivity and the status of subspecies populations in the wild, priorities for additional captures are: (I) up to about 20 animals from the four most endangered black subspecies; (ii) two or three *C.s. cottoni,* perhaps over several years; (iii) *D.b. minor* and *bicornis,* about 10 animals each from a range of locations; and (iv) several *C.s. simum* and *D.b. michaeli* from regions not well-represented currently. Fortunately, with the possible exception of C.s. *cottoni* from Garamba, capturing additional wild rhinos raises little conflict between the needs of the wild and the needs of the captive populations. Most wild subspecies' populations are either large and stable enough to withstand

the removal of several animals without harm, or so small and imperil lied that they seem doomed even without any removals. it is the intermediate situation, where removals would decrease the stability of the wild population, where a dilemma arises (Maguire, 1986).

DECISION ANALYSIS ——MIXED CAPTIVE POPULATIONS OF BLACK RHINOS

Preliminary versions of two decision analyses of aspects of the captive management of black rhino subspecies were presented for discussion. The management questions addressed were: (I) which subspecies should be included in the captive program, and at what population sizes; and (ii) why choose captive management in zoos over semicaptive (intensive *In situ)* management of the most endangered subspecies?

Which black rhino subspecies, and how many?

The decision problem addressed by this analysis is how to allocate the approximately 220 captive spaces potentially available for black rhinos (assuming an equal number of captive white rhinos) among the seven extant subspecies. The alternatives considered are: PLAN 1 ——a mixed population of the four most endangered subspecies *(ladoensis, chobiensis, brucii,* and *longipes)* of about 100 animals, and about 40 each of *bicornis, minor* and *michaeli;* PLAN 2 —— a mixed population of the four most endangered subspecies of about 220 animals; and PLAN 3 ——two captive populations consisiting of about 110 animals each of—*minor* and *michaeli.* The major uncertainty affecting how well each of these plans might serve the needs of rhino conservation is survival of *bicornis, minor* and *michaeli* in the wild. The first plan stresses including all subspecies in the captive program, even if some must be held in mixed populations, or in smaller management units than would be desirable in the tong term. The justification for maintaining *bicornis, minor* and *michaeli* at only 40 animals each is optimism about exchanging animals with surviving wild populations of these subspecies. The second plan emphasizes using available captive space to enhance the survival of those subspecies least likely to survive in the wild; it also assumes some optimism about the survival in the wild of the other three subspecies. The third plan concentrates on managing those subspecies currently represented In the captive population, probably with the addition of new founders for *minor,* at least. It emphasizes using the captive plan to bolster the subspecies with the best prospects for survival in the wild. It reflects a more pessimistic (some may want to say realistic) view of what can reasonably be accomplished by rhino conservation efforts. The subspecific groups used in this analysis correspond roughly to the four management units proposed in the workshop recommendations, with the southwestern unit being *bicornis* (and *chobiensis?),* the southern-central unit being *minor,* the eastern unit being *michaeli,* and the northern-western unit being *brucii, ladoensis* and *longipes.*

Objectives and criteria

The criteria for weighing which plan is best reflected several rhino conservation objectives. First, to maximize the number of distinct “lines”, or subspecies, surviving. This objective recognizes local adaption of geographic populations and coadaptation of gene pools. Second, to maximize the genetic variation represented by the founder populations for the captive program. This objective emphasizes the role of captive propagation in conserving the raw material for adaptation, genetic diversity. In calculating the proportion of genetic variation from the wild population represented by each subspecies, I assumed that the subspecies share one-half of their variation, which is probably an underestimate. Initial variation, rather than retention of variability, was used as a criterion because in the short time scale of 15 years, little or no variability is lost. The third objective is to minimize the rate at which variability is lost from the captive population due to drift and inbreeding. The concern here is that a captive plan should be tenable over the long, as well as the short, term, except in situations where the peril to a species is clearly temporary. In calculating the inbreeding coefficient for each plan, expressed as the percent variation lost per generation, I have assumed that the effective population size for captive populations will be about half the census size, which may be a little optimistic; that the effective population size of each of the surviving wild populations will be about 30, due to the fragmented nature of these populations and the lack of genetic management in semicaptive populations; and that there will be continued interchange among captive and surviving wild populations, so that the total effective population size for each subspecies is the sum of the captive and wild segments. The final objective is to minimize dollar costs. Since the same number of captive rhinos will be managed in each plan, the major difference among them is in the cost of acquiring new founders. The cost estimates are based on capturing about 20 of the northern-western animals for plans 1 and 2, about 10 *bicornis* for plan 1, and about 10 each of *minor* and *michaeli* for plans 1 and 3.

Decision tree

The preceding information about the three management alternatives, sources of uncertainty, and evaluation of outcomes according to the four decision criteria is represented in graphical form on the decision tree in Figure 1. The decision points are represented by squares, with branches of the tree for each management alternative. The sources of uncertainty are represented by branches emerging from circles, denoting random nodes, where the manager has no control over events. The values of the decision criteria associated with each combination of management action and random event (survival or extinction of *bicornis, minor* and *michaeli)* are listed to the right of the corresponding branches of the tree.

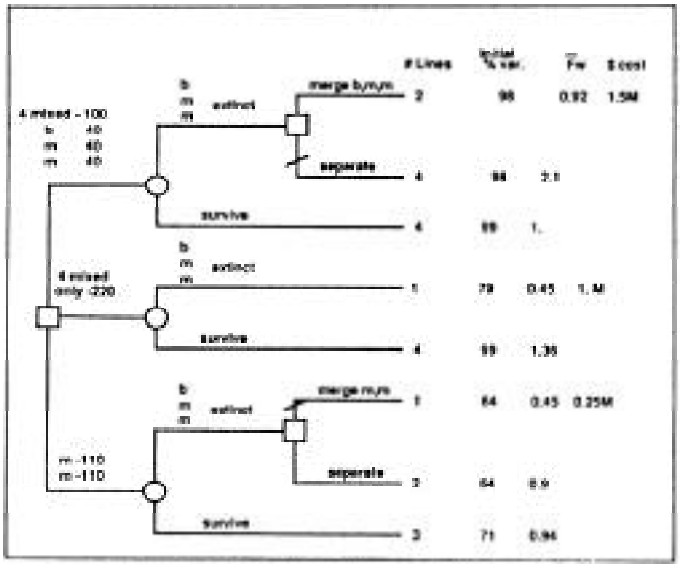

Figure 1. *Decision tree for analyzing how many black rhino subspecies to include in the captive program and at what population sizes. Fw = inbreeding coefficient; b, m, m* = bicornis, minor *and* michaeli.

Two additional decision points have been added on the branches representing plans 1 and 3. If *bicornis, minor* and *michaeli* go extinct in the wild, merging the captive populations of these subspecies may be desirable to maintain a larger effective population size for each management unit. For simplicity, I have assumed that this merger *will* take place under plan 1, reducing the inbreeding coefficient in the captive population from 2.1 to 0.92, but not in plan 3, where the separate populations are large enough to have an inbreeding coefficient less than 1 percent (which is often used as a maximum in breeding programs). The rejected alternatives are denoted by slashes on the corresponding branches of the tree (Figure 1).

Analysis of the tree

The first thing to notice from the decision tree is that for two of the decision criteria, dollar cost and initial genetic variation, there is no decision problem. One alternative is clearly best, regardless of the survival of *bicornis, minor* and *michaeli:* plan 1 for initial variation and plan 3 for dollar cost. Note also that for number of lines surviving, plan 1 always doss at least as well as any other plan, regardless of the random event, with 4 lines surviving if *bicornis, minor* and *michaeli* survive, and 2 lines surviving if they do not, i.e. plan 1 dominates the other plans for this criterion. In contrast, which plan is best for minimizing inbreeding depends on the random event: if the three survive in the wild, then plan 3 is best (0.94), but if they go extinct, then plan 2 is best (0.45).

To determine which plan is optimal in an uncertain environment, the probabilities of survival and extinction of *bicornis, minor* and *michaeli* are used to weight the criteria associated with the possible outcomes. These weighted values, or expected values, of the four criteria are listed in Table 1 for three estimates of probability of extinction, which may be viewed as ranging from optimistic (0.25) to pessimistic (0.75). the principles of decision theory suggest that the best decision under uncertainty is the one with the best expected outcome. For each of the four criteria, the optimal decision is starred in Table 1. Note that for pessimistic views of extinction in the wild, plan 2 minimizes Inbreeding whereas, for optimistic views, plan 3 is best.

Tradeoffs

The major remaining hurdle in selecting one option as superior is resolving tradeoffs among the four criteria. Maximizing number of lines and maximizing initial variation conflict with minimizing dollar cost and minimizing inbreeding, In some instances, it is easy to resolve these tradeoffs informally. For example, it seems obvious that plan 1 is superior to plan 3, for number of lines, initial variation, and inbreeding, because a small increase in inbreeding nets a large increase in the other two criteria. Other tradeoffs may not be obvious: is it worth half a million dollars to raise initial variation from an expected value of 89 to **98.5%** (for p = 0.5)? Structured series of questions can be used to help managers articulate how much of one criterion they are willing to sacrifice to gain in another dimension (Maguire, 1986; Behn and Vaupel, 1982). As an initial strategy, I propose capturing as much of the genetic variation as financial constraints allow (plan 1), coping with inbreeding later by merging the captive populations as necessary. Meanwhile, studying the structure of genetic variation among geographic populations, in conjunction with the capture program, will help reduce uncertainty about outbreeding depression or loss of coadaptation in merged populations.

Table 1. Expected values of the four decision criteria for each of the three management alternatives from Figure 1, calculated for three values of p = probability that bicornis, minor *and* michaeli *become extinct in the wild. Fw = inbreeding coefficient. The best expected values for each criterion and each value of p are starred.*

	Expected values			
Alternative	No. Lines	Initial Var.	Fw	$(M)
	P =0.25			
mixed, b, m, m	m 3.5*	98.75*	0.98	1.5
mixed only	3.25	94	1.13	1
m, m	2.75	69.25	0.93*	0.25*
	p = 0.5			
mixed, b, m, m	3*	98.5*	0.96	1.5
mixed only	2.5	89	0.905*	1
m, m	2.5	67.5	0.92	0.25*
		p = 0.75		
mixed, b, m, m	2.5*	98.25*	0.94	1.5
mixed only	1.75	84	0.68*	1
m, m	2.25	65.75	0.91	0.25*

CAPTIVE VERSUS SEMICAPTIVE MANAGEMENT OF THE MIXED POPULATION

The preceding analysis argued in favour of starting a mixed captive population of the four most endangered subspecies, as a means of capturing and retaining genetic variation represented (temporarily) by the surviving remnants of these subspecies. Why should this mixed captive population be maintained in zoos, rather than in a semicaptive situation? Why should the four subspecies be mixed, rather than being maintained separately?

In a decision analysis to address these questions, four alternatives were considered. First, controlling poaching and protecting habitat for these subspecies in the wild: the major uncertainty here is whether these severely fragmented populations will survive even with increased protection. Second, maintaining a mixed, semicaptive population of about 100 animals: the major uncertainty is the possible impact of outbreeding depression on effective population size. I assumed that if outbreeding depression is a problem, the ratio of Ne to N may be as low as 0.05, rather than the 0.2 assumed for semicaptive management in the absence of outbreeding depression. The third alternative is maintaining separate zoo populations of the four subspecies with about 25 animals each: the major uncertainty is whether there will be sufficient founders to successfully establish each subspecies in captivity. The fourth alternative is a mixed zoo population of about 100 animals: the major uncertainty is that outbreeding depression could reduce the Ne to N ratio from about 0.5 to perhaps 0.1. The same four criteria were used to evaluate possible outcomes as in the preceding analysis.

Decision tree

These alternatives, uncertainties and criteria are represented by the decision tree in Figure 2. The dollar costs are listed on the decision branches, rather than to the right of the tree, In assigning probabilities to random events, I have assumed that the fates of the wild population of different subspecies are independent (which may not be the case if the same factors are leading to decline everywhere), and that the

probability of a single subspecies surviving in the wild *for* 15 years is 0.16. Similarly, in assigning probabilities for success in establishing separate captive populations, I have assumed that the different subspecies behave independently (which may not be the case if the same husbandry problems afflict all), and that the probability of successful establishment of a single subspecies is about 0.1 (mainly because of the difficulty in obtaining sufficient founders; only about half the wild caught rhinos have bred in captivity). I have assumed that the probability of outbreeding depression In the mixed populations is about 0.1, which, given the small differences among subspecies observed in genetic studies so far, may be an overestimate. The probabilities are listed on the random events branches in Figure 2.

In calculating the inbreeding coefficients from effective population size, I have assumed an Ne for surviving wild populations of 3, which is probably realistic for these very fragmented remnants, in assigning initial variation captured by different plans, it is not strictly true that 20 wild founders would capture 100% of the variation from the wild population, but this figure is close enough for comparison with the other alternatives and it simplifies calculations.

Analysis of the tree

In examining the decision tree, notice first that the mixed zoo and mixed semicaptive populations offer the "certainty"

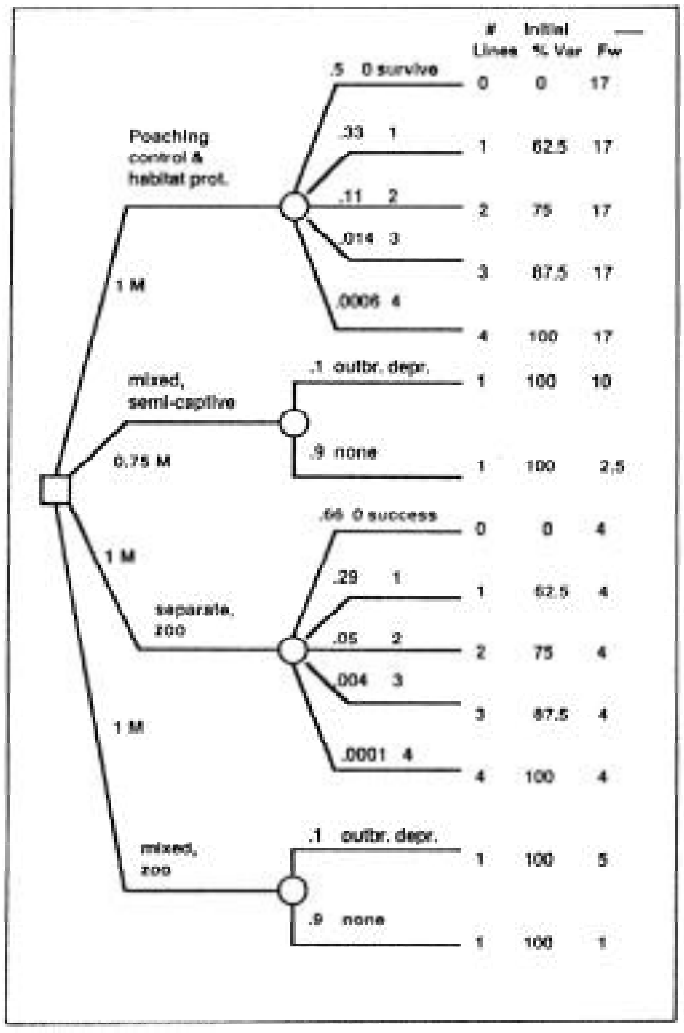

Figure 2. Decision tree for analyzing management alternatives for conserving the four most endangered black rhino subspecies (ladoensis, longipes, brucii, *and* chobiensis). *Dollar costs of each alternative are listed on the decision branches.*

Table 2. *Expected values for the four decision criteria for the four management alternatives in Figure 2.*

	Expected Values			
Alternative	No. Lines	Initial Var.	Fw	$(M)
Poaching control w/ habitat protection	0.63	38	17	1
Mixed, semicaptive	1*	100*	3.25	0.75*
Separate, zoo	0.4	26	4	1
Mixed, zoo	1*	100*	1.4*	1

that one line will survive against the gamble that from 0 to 4 lines might survive in the wild or in separate zoo populations. Which alternative is best depends on the probabilities for the uncertain events affecting survival In the wild and successful establishment of separate captive populations.

The expected values for each of the four decision criteria for the probabilities listed on the decision tree appear In Table 2. Notice that the probabilities of survival for the wild populations and of successful establishment of separate captive populations.

The expected values for each of the four decision criteria for the probabilities listed on the decision tree appear in Table 2. Notice that the probabilties of survival for the wild populations and of successful establishment of separate captive populations are so low that the expected values for number of lines surviving are much lower for plans 2 and 4 than for the mixed population options. The major advantage zoo management has over semicaptive management is the lower rate of inbreeding under more intensive management. Greater attention to manipulation of breeding stock and exchange of animals along semicaptive populations could reduce this advantage of zoos and make semicaptive management more attractive. The thrust of the captive program is to maintain genetic variation that would otherwise be lost; at present, zoos are best equipped to do this although there is certainly room for improvement in zoo management as well.

CRITIQUE OF ANALYSES AND RECOMMENDATIONS FOR FUTHER WORK

The advantages of decision analysis for endangered species management are discussed by Maguire (1986). in these examples, the conclusions (that all subspecies of black rhino should be included in the captive program, and that a mixed population of the most endangered subspecies should be maintained in zoos) should be taken less seriously than the form of the analyses, since they are based on very preliminary information. The formal decision tree structure helps to organize and display information pertinent to these rhino management problems. Scrutiny of the decision tree helps to identify which management alternatives are best under any conditions, and which depend critically on chance events; alternatives with "certain" outcomes can be distinguished from risky ones.

Sensitivity analyses, showing how the decision might be affected by changes in the probabilities assigned to random events or by the values assigned to the decision criteria, are important for building confidence in a particular course of

action or directing further study. in the first example, different probabilities of extinction of *bicornis, minor,* and *michaeli* were used to show how the decision strategy might change. In the second example, sensitivity of the decision to the probabilities of outbreeding depression, of survival in the wild, and of successful establishment of separate captive populations can help identify the circumstances under which semicaptive management would be better than zoos.

A structured analysis shows where additional information about chance events could reduce uncertainty and lead to a better decision. Genetic analyses of rhino subspecies can help reduce uncertainty about outbreeding depression in mixed populations, guiding the sampling of geographic regions for founders of captive and semicaptive populations and the merging of these populations in the future.

Tradeoffs among conflicting criteria, particularly between financial and biological criteria, are typical of endangered species management decisions. The two examples presented here raise the difficult question of how the value of obtaining founder animals from the northern-western subspecies of black rhino should be weighed against the difficulty and expense of doing so.

in addition to the two questions addressed by these preliminary examples, many other rhino management decisions might benefit from formal analysis:

(i) Under what circumstances is wild, intensive *In situ,* or *ex situ* management best? Among the criteria to be used for this decision are: biological impacts, including disruption of behavioural adaptations or coadapted gene pools; political impacts on local and national support for conservation; socio-economic impacts on local economies; and likelihood of sub-species survival.

(ii) How many founders are required to justify maintaining a separate subspecies population? At what point should some subspecies populations be merged for semicaptive or captive management? Among the issues here are the genetic and demographic risks of few founders weighed against the irreversibility of merger.

(iii) What are the optimal strategies for translocating animals among semicaptive and/or captive populations? Which sexes and ages should be moved, what size groups, how frequently? The concerns here are the relative genetic and demographic contributions of different sexes and ages, social disruption caused by moving animals, risks of mortality during and after translocation, financial cost, and hazards of inbreeding in isolated populations. Some of these issues are addressed in Maguire (1986) and in previous analyses of translocations to augment grizzly bear populations (Maguire, unpublished report to U.S. Forest Service).

(iv) What are the risks and benefits of ongoing exchanges of animals, or genetic material, among captive, semicaptive and wild populations? Social disruption, impact of removals, transmission of disease, risks of injury or death to individual animals, disruption of local adaptation, and loss of genetic variation from drift and inbreeding are among the considerations here.

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SMALL POPULATION MANAGEMENT OF BLACK RHINOS

Session Chairman DAVID CUMMING

STATUS OF BLACK RHINOS IN THE WILD

The black rhino has declined more rapidly over the past 20 years than any other large mammal. In 1970 there were about 65 000 black rhinos in Africa; the total is now under 4 000, a decline of 94%. The population sizes in the various African countries within this decade are roughly as shown in Table 3. The remnants of a number of the populations are scattered as individuals or in very small groups over vast areas. For instance, the estimated 200 rhinos remaining in the Selous Game Reserve of Tanzania are dispersed over 55 000 km^2.

The recent decline of the species is due almost entirely to commercial poaching for rhino horn. The decline in South Africa, due to natural factors in the Umfolozi-Hluhluwe complex, appears to be the one exception (the 1984 figure was probably an overestimate). In the early 1980's about half of the horn put onto the world market went to North Yemen where it is used for making dagger handles, while the remaining half went to eastern Asia for the production of traditional medicines. Most of these rhino horn mixtures are produced because they are believed to lower fevers, not because of alleged aphrodisiac properties. North Yemen has recently strengthened some controls on the import and use of rhino horn, so there may be changes in the relative importance of the markets.

Prices for African rhino horn have risen from about $30 per kg wholesale in 1970 to about $900 per kg today. Asian rhino horn is believed to have more potent medicinal properties and therefore commands much higher prices in eastern Asia. To halt and reverse the precipitous decline in the numbers of black rhinos will require concerted action by many individuals and organisations. International, national and local conservation efforts will be most effective and make the best use of scarce resources if they are part of a planned campaign. To achieve this coordination of effort, a broad framework of policies on rhino conservation (i.e. a continental rhino conservation strategy) must be agreed upon by the principal agencies involved, and plans of action ——with clear priorities ——must also be elaborated in line with—these policies, and kept updated as the black rhino situation changes. The African Elephant and Rhino Specialist Group (AERSG) is currently developing a continental black rhino conserva-

Table 3. Status of black rhinos in Africa.

	1980	1984	1987	% of total 1987 rhino population
Tanzania	3 795	3130	270	7%
C.A.R.	3 000	170	10?	0.2%
Zambia	2 750	1650	110	3%
Kenya	1 500	550	520	14%
Zimbabwe	1 400	1 680	1 760	46%
South Africa	630	640	580	15%
Namibia	300	400	470	12%
Sudan	300	100	3	—
Somalia	300	90	?	—
Angola	300	90	?	—
Mocambique	250	130	?	—
Cameroon	110	110	25?	0.7%
Malawi	40	20	25	0.7%
Rwanda	30	15	15	0.4%
Botswana	30	10	10	0.2%
Ethiopia	20	10	?	—
Chad	25	5	5?	—
Uganda	5	—	—	—
TOTAL	**14 785**	**8 800**	**3 800**	

tion strategy, and has been producing annually-revised action plans for the conservation of rhinos and elephants.

In discussing the draft strategy, an emphasis that emerged from the workshop was the need for interactive management of wild and captive populations in order to maintain genetic variability. However, it was agreed that *ex situ* breeding programmes should avoid mixing rhinos from different regions of Africa in order not to destroy probable adaptations to particular environmental factors in these ecologically divergent regions. The numbers of remaining rhinos in the four regional groups that were identified for separate genetic management are shown in Table 4.

Table 4. Estimated numbers of black rhinos in regional units.

Regional conservation unit	Number
Southwestern	500
Southern/Central	2 600
Eastern	600
Northern/Western	50

STATUS OF BLACK RHINOS IN CAPTIVITY

Tables 5 and 6 summarize the current status of black and other rhinos in captivity at the time of the workshop. Figures differ slightly from those used by Lynn Maguire and Robert Lacy in their analyses in these proceedings—owing to different sources of information—but not to a significant extent. There appears to be captive habitat in zoos for about 700-800 rhinos, using current collections as a crude estimate. Black rhinos are currently allocated about 20% of these spaces, while white rhinos occupy a disproportionate 60% (owing largely to their ready availability from South Africa). The black rhino population in North America, now under management of the AAZPA Species Survival Plan (SSP), has been increasing slowly over the last five years at a rate of about 2% per annum (Table 7). Birth rates have been quite encouraging (in contrast to the white rhinos, which have not reproduced well as a probable consequence of this species' inclination to breed better in group situations than when kept

Table 5. Current populations of rhino in captivity. Sources are AAZPA Species Survival Plans (SSP), the international Species Inventory System (ISIS), International Zoo Yearbook (IZY), and the International Studbooks for African Rhinos (Zoo Berlin) and Indian Rhinos (Basel Zoo).

Species	North America	World	
		IZY	Studbook
Black	30/38 =68	68/80 = 148	82/98 =180
White			
Southern	70/93 = 163	177/215 = 392	313/357 = 670
Northern	1/0 = 1	6/5 = 1	6/5 = 1
Indian	16/12 = 28	44/35 = 79	44/35 = 79
Sumatran	0	3/6 = 9	3/6 = 9
Javan	0	0	0
TOTAL	117/143 = 260	298/341 = 639	448/501 = 949

Table 6. Estimated captive capacity or habitat (space and resources) for rhinos in the world's zoos.

Species	North America	World
Black	125	200-250
White	100 (+25?)	200-250
Indian	75	150
Sumatran	75	150
Javan	?	?
TOTAL	375-400	700-800

Table 7. Performance of North American zoos with black rhinos, 1982-1986.

Year	Births	Deaths	Dispersed	Imported
1982	1/3	2/2		1/1
1983	2/2	0/1		2/0*
1984				
1985	2/5	3/2	0/1	
1986	4/3	3/3		
TOTAL	9/13	8/8	0/1	3/1

*Captive born in Japan

as pairs). Death rates in black rhinos have been high, largely because of the haemolytic anaemia syndrome discussed later in these proceedings. intensive research to resolve this problem is in progress and some hopeful insights have already been obtained, especially in terms of possible vitamin E deficiencies.

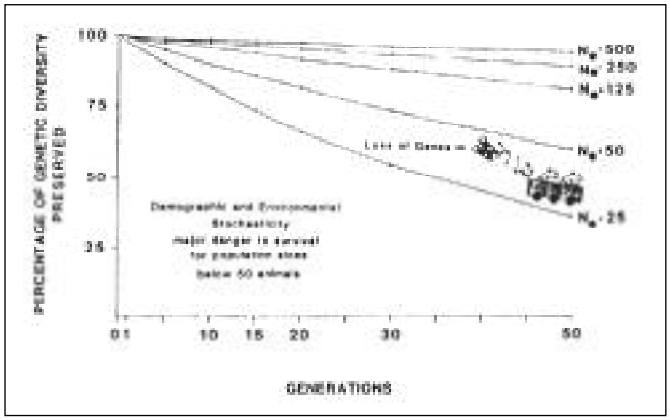
LONG-TERM MANAGEMENT OF SMALL RHINO POPULATIONS

Thomas Foose (American Association of Zoological Parks and Aquariums).

Overview of concerns

As discussed by Lynn Maguire in the preceding session, and elaborated by Robert Lacy in the following presentation, the trend towards very small and fragmented populations in the wild (i.e. towards the situation of rhinos to captivity) makes these populations vulnerable to extinction for genetic and demographic reasons. Small populations lose genetic diversity rapidly at the population level (Fig. 3) as well as at the individual level. At the population level, genetic diversity Is

Figure 3. The decline of genetic diversity (measured as average heterozygosity in the total population) over 50 generations for various effective population sizes (Ne), possible for a total population (N) of 250.



vital to permit adaptation to continually changing environments. At the individual level, genetic diversity is required to maintain the "vigour" of the animals; loss of diversity in individuals is known as inbreeding and a consequent decline in survival and fecundity rates is inbreeding depression.

Conservation biologists have suggested that genetically effective population sizes (Ne's) of 50 or more are necessary for the shorter term (5-10 generations) mainly to counteract inbreeding depression, while Ne's of 100-500 or even more may be necessary over the longer term to maintain adaptability. The vulnerability of small populations to demographic risks (disease epidemics, natural disasters, uneven sex ratios, etc.) imposes a further minimum limit to desirable population size: conservation biology—models suggest that populations must be no smaller than 25-50 total individuals to survive unpredictable (stochastic) demographic risks.

To preserve a species against these genetic and demographic risks, it is therefore necessary to establish some *minimum viable population size* (MVP). The actual MVP that is recommended will depend on the defined objectives for the species at risk as well as the biological characteristics of that species (Soule *et al.,* 1986). The major relevant concerns are as follows.

1.) The probability of survival of the population. No finite population size will completely insure a species against stochastic extinction, but It is sometimes possible to specify population sizes that will insure some probability of survival (e.g. 50%; 90%). For some given period of time, the higher the stipulated probability of survival, the larger the MVP required. 2.) The level of genetic diversity to be preserved. Obviously, the top objective would be to retain all the genetic diversity. However, with the restricted populations possible (In the wild or captivity), something less than all may have to be accepted for some period of time. Preserving rarer alleles (i.e. specific varieties of genes) will require larger MVP's than merely maintaining average heterozygosity (some variation of any, non-specific kinds). Preserving 95% of average heterozygosity will require an MVP twice as large as'90% will. Population geneticists are not certain how much genetic diversity is enough but levels of at least 90% of average heterozygosity have been strongly suggested.

3.) How long this level of genetic diversity must be preserved. The optimal answer is indefinitely, i.e. the species could then continue to evolve as environments change. But again, there may have to be compromises. Hopefully, intensive programmes will be needed only through the present ""demographic winter"' (the period extending for the next 200 to 500 years, during which human population growth and development will continue and intensify disruption of natural systems). However, the winter may vary on a species-by-species and area-by-area basis.

Biological characteristics that influence MVP sizes include the following:

1.) The generation time of the species. Genetic diversity is lost generation by generation, not year by year. Thus some given period of time, e.g. 200 years, represents more generations, hence more opportunity to lose diversity, for a species like a galago than it does for a species like a rhino.

2.) The Ne/N ratio of the population. Loss of diversity does not depend simply on total population size, but rather on the genetically effective size (which reflects how the animals are actually reproducing to transmit genes to the next generation). Very generally, the genetically effective size of a population depends on:

— the number of animals actually reproducing;
— the sex ratio of the reproducing animals;
— the relative lifetime number of offspring (i.e. family size of animals in the population).

Since Ne is normally less (often much less) than the census number (N), MVP's must be larger than the population sizes prescribed by genetic calculations since these prescriptions are always in terms of Ne.

3.) The number of founders that establish a population. Founders are animals out of a wild population that are used to establish a captive or a new wild population (or augment a recovering wild population). Conversely, they could be animals from captivity that are used to re-establish a species in the wild. in general, the larger the number of founders, the smaller the MVP needed for some genetic objectives. However, there is a point of diminishing returns so that usually 20-30 founders may be adequate.

4.) The reproductive rate or recovery potential of the population. Much genetic diversity can be lost either as the population grows from its foundation size to carrying capacity or during recovery from periodic reductions. in general, the higher the reproductive rate and hence growth or recovery to carrying capacity, the less genetic diversity is lost.

5.) The degree of subdivision or fragmentation in the population. If a species is fragmented into a number of subdivisions which are Isolated from one another, animals may not be able to move around for breeding and hence exchange of genetic material. Such situations can cause loss of genetic diversity. On the other hand some subdivision may assist retention of some kinds of genetic diversity. The important point is that conservationists must analyze the genetic processes in the species under consideration and develop an appropriate management plan that may include artificial movement or manipulation of animals, to synthesize many separate smaller populations into a so-called metapopulation capable of greater long-term viability.

Clearly, there is no single MVP figure that will apply to all species or to all situations for any given species. Rather, MVP's will vary depending on the objectives of the program and the circumstances of the species. Detailed explanation and expansion of the MVP concept are provided by Gilpin and Soule (1986), Shaffer (1987) and Soule (1987). The process of determining the size of a population that is required to achieve some level of genetic and demographic security has come to be known as *population viability analysis (PVA).*

PVA attempts for black rhinos

Table 8 represents some initial attempts at prescribing MVP's for both wild and captive black rhinos. These analyses were performed using microcomputer software developed by Jon Ballou of the National Zoological Park in Washington, DC, and are extremely tentative. To refine the PVA models and their data inputs, there needs to be more collaboration between conservation biologists and field managers of black rhinos. However, since there is an urgent need for management guidelines, a number of preliminary recommendations based on these rough analyses have been generated for consideration.

An Ne = 500 is proposed for each regional conservation unit of black rhinos. This represents a number sufficiently high to ensure maintenance of genetic diversity (e.g. 90% average heterozygosity for 50 rhino generations) and demographic security.

An Ne/N ratio of 0.25 to 0.5 is proposed as a further operational guideline in formulating conservation strategies for black rhinos. With management, especially in captivity, it may be possible to improve this ratio. Simple arithmetic indicates that to achieve an Ne = 500 with a worst case situation of Ne/N = 0.25, an MVP of 2 000 would be required for each conservation unit of rhinos.

Since black rhino populations will be fragmented and resources for conservation limited, it also seems advisable to suggest a size for individual populations of black rhinos within each conservation unit. The number roughly indicated by analyses so far is 100-200. This guideline does not dictate that populations smaller than this size are worthless but that they should probably receive lower priority for conservation efforts than larger ones. Realistic cost-benefit analyses need to be performed on each of the rhino populations of limited viability to determine if intensive and interactive management in feasible in both logistic and economic terms, it should be emphasized that the figure suggested here applies not to actual current population, but to potential size of the population in the given area if rhinos can be adequately protected to reach carrying capacity.

Finally, it should be realized that individual populations of 100-200 are not likely to be genetically and demographically viable by themselves over periods of time in the order of centuries. There will need to be interchange between separate populations to create the so-called "metapopulations" for each conservation unit. Where natural migration is not possible between separate populations, management will have to artificially move animals for genetic and demographic reasons as suggested by appropriate PVA analyses.

Because of the limited space and resources available in ex *situ* facilities, MVP's may have to be, and probably can be, even more precisely defined for captive than for wild populations. An objective for captive propagation of preserving 90% of average heterozygosity for 200 years is a common recommendation of conservation biologists considering principles of population genetics (i.e. inbreeding) and demography as well as the likely period of time that human pressures will be most intense on wildlife. To achieve objectives of preserving a significant fraction (90%) of the wild gene pool for 200 or so years, a number of combinations of ultimate carrying capacity, initial founder numbers, and population growth rates will produce the desired results (as demonstrated in Table 8).

As a result of these preliminary analyses, the zoo community is proposing to develop captive populations of 150 each for at least two of the conservation units of black rhinos; the North American AAZPA SSP will attempt captive populations of 75 for each of these two units. The constraints imposed by the biological characteristics of the species will prescribe a critical minimum for the number of founders (i.e. animals out of the wild) that will be needed to establish the captive population. For black rhinos, 20-25 effective founders for each conservation unit maintained seems desirable.

Table 8. Minimum viable populations required to preserve 90% average heterozygosity for various periods, in several demographic situations.

A. GENERATION TIME = 15 YEARS.
POPULATION GROWTH RATE = 1.03/YEAR
Ne/N Ratio = 0.5

		YEARS						
		75	150	225	300	450	600	750
EFFECTIVE	10	–	–	–	–	–	–	–
NUMBER	20	62	131	236	367	603	8911	134
OF	25	50	121	189	273	459	641	832
FOUNDERS	30	50	103	170	241	393	551	712
	50	50	100	156	203	319	439	561
	75	50	100	150	193	297	404	513
	100	50	100	150	193	289	392	495

B. GENERATION TIME = 15 YEARS. POPULATION GROWTH RATE = 1.06/YEAR
Ne/N Ratio = 0.5

		YEARS						
		75	150	225	300	450	600	750
EFFECTIVE	10	115	292	534	786	1310	1842	2384
NUMBER	20	50	115	187	261	414	568	727
OF	25	50	106	170	235	369	505	642
FOUNDERS	30	50	102	160	221	345	471	598
	50	50	100	147	200	308	417	527
	75	50	100	150	193	293	397	501
	100	50	100	150	193	289	389	489

C. GENERATION TIME = 15 YEARS.
POPULATION GROWTH RATE = 1.06/YEAR
Ne/N Ratio = 0.25

		YEARS						
		75	150	225	300	450	600	750
	10	230	583	1069	1573	2621	3685	4769
EFFECTIVE	20	101	231	374	522	829	1136	1451
NUMBER	25	100	212	339	470	737	1010	1284
OF	30	100	204	320	442	689	942	1195
FOUNDERS	50	100	200	295	400	615	835	1054
	75	100	200	295	386	589	794	1001
	100	100	200	295	386	579	778	997

FURTHER GENETIC AND DEMOGRAPHIC ANALYSES OF SMALL RHINO POPULATIONS

Summary of presentation by Robert Lacy (Chicago Zoological Society)

This work is quite preliminary, providing initial insights and possible directions for future analysis, not definite conclusions or recommendations about rhino populations. The

analyses were conducted using best-guess data available from a variety of sources; the data, the models used, and the analyses of the results can and should be improved.

Analysis of founder members for the captive populations

Captive populations often derive from so few wild-caught "founders" that they poorly represent the genetic (and morphological, ecological, physiological, and behavioural) diversity of the wild populations. To examine the founder stock from which the captive populations of African rhinos descend, I analyzed the international studbooks for black and white rhinos (updated computer versions provided just prior to the October 1986 workshop). Numbers of living wild-caught animals, numbers of founders, and numbers of""effective founders" were calculated.

Founders were defined as wild-caught animals (currently alive or not) that have living descendants in captivity. Thus, if a wild-caught animal left no living descendants, it is not a founder of the captive population. Even if a wild-caught animal is still alive, but has not left any progeny, it is still not a founder but rather is a"*potent/al* founder, of potential genetic value but so far just an occupant of valuable space for breeders.

Effective founder number is a measure that I devised to account for unequal representation of founders in the gene pool of the present population, it is analogous to the concept of ""effective number of alleles"' at a genetic locus, and related to the concept of ""effective population size". Algebraically, the effective number of founders is

$1/(P1^2 + P2^2 + ... + Pn^2)$,

In which Pi is the proportion of the captive (and non-wild-caught) gene pool that has descended from founder I. The Pis are the founder representations calculated from pedigree data and often discussed in studbook management. If the founder representations are all equal, then the effective number of founders will equal the actual number of founders. If founders have contributed unequally, the effective number will be less. For example, if three founders have contributed 50%, 25% and 25% to the living captive population, the effective number of founders would be 2.67. If one founder contributes 50% of the gene pool, and a very large number of founders each contributes a small fraction of the other 50 %, then the effective number of founders approaches 4. The effective number of founders can be thought of as the number of ideal (equally contributing) founders that would be required to obtain a population with the genetic diversity represented in the actual population. Bottlenecks in the pedigree can alter this somewhat, because they make it more likely that the entire genetic contribution of a founder derives from only half its genes. in the case of rhinos, however, bottlenecks exist only in the lineages of poorly represented founders, and therefore affect the effective number of founders almost not at all.

The results of the analyses of studbook data are as follows.

BLACK RHINOS

World captive population:

87 males (38 wild-caught, 20 captive born)

103 females (55 wild, 48 captive born)

82 identifiable founders (12% of captive animals are of unknown parentage and source, thus more founders may exist)

49.6 effective founders.

North American population:

32 males (12 wild, 20 captive born)

41 females (19 wild, 22 captive born)

(17% are of unknown history)

35 identifiable founders

24.6 effective founders

WHITE RHINOS

World captive population:

309 males (195 wild, 114 captive born)

348 females (259 wild, 89 captive born)

(28% are of unknown parentage)

121 identifiable founders

17.6 effective founders

(Male No. 52 contributed 11% of current gene pool) North American population:

86 males (53 wild, 33 captive born)

113 females (74 wild, 39 captive born)

(21 % are of unknown history)

47 identifiable founders

16.1 effective founders

(Male No. 52 contributed 16% of gene pool)

The captive populations have enough effective founders to be sufficiently representative of the gene diversity in the wild. However, most founders have contributed very little, a few founders have left many descendants, and about a third of the black rhinos and about half of the while rhinos in captivity are wild-caught animals that have never bred. Thus, the captive population should be in reasonable shape genetically, but a large number of wild-caught animals have been wasted with respect to genetic and demographic goals of captive breeding.

Analysis of demographic stability of small populations of rhinos

Even if a population is growing, on average, random fluctuations in births and deaths can lead to chance extinction of a small population. Once a population has grown to large size, such chance extinction is unlikely. I used a population stimulation program written by James Grier of North Dakota State University to examine the likelihood of success (non-extinction) of rhino populations started from small numbers of founders. The intent was to provide some rough guidelines for the re-establishment of populations in reserves. The simulation model very optimistically assumes that births and deaths are random processes that occur with some constant probability in each year. Thus, fates of individuals are independent; good years and bad years are due to accidental concordance between reproduction and mortality within the population; no environmental fluctuations exist that would cause population-wide trends in reproduction and mortality. Because environmental fluctuations do exist in the wild and do affect populations as a whole, the results below should be thought of as upper limits on the likelihood of a small population persisting.

The demographic parameters input into the model were obtained from field data on East African black rhino populations, gleaned from AERSG reports, reports of Peter Jenkins to Kenyan authorities, and other published and unpublished sources. Rhinos were assumed to be capable of breeding at

age 7, with each adult female producing offspring in 28% of the years (3.57 year average interbirth interval).

Juvenile (first year) mortality in the wild has been reported be about 16%, with 5 to 10% annual mortality of adults. I explored the models with 13%, 15%, 16%, or 20% juvenile mortality, and 5%, 60/a, or 6.808% adult mortality. This last value of adult mortality would lead to a stable, non-growing population when juvenile mortality was 160/a. Higher values of adult mortality were not modelled (even though higher values have been recorded in the field), because they would lead to precipitous declines in the population and thus extinction of the population would be virtually certain. Either 10 or.20 animals were used to begin each simulated population, and populations were followed for 85 or 170 years (5 or 10 generations). Table 9 gives the expected reproductive rates (Ro, population growth per generation, determined from life table analysis, not from the simulation program), the percent of the simulated populations (out of 100 in each case) that did not go extinct in the time span considered, and the average population size at the end of the simulation of those populations that survived. Not all combinations of parameters were tested.

Over the time span considered, random fluctuations in births and deaths would lead to the extinction of relatively few populations of rhinos that have long-term average growth rates greater than one. However, the field estimates of birth and death rates, if accurate, mean that rhino populations have very low net reproductive rates (not a surprise), and that even slight increases in deaths or decreases in births will lead to long-term decline rather than population growth. Note that the claimed rates of population growth in some game reserves (e.g. those in South Africa) do not seem compatible with the reported birth and death rates that were used in this model.

To a considerable extent, the high rate of population survival in this model results from the short time span considered and the lack of any limit to population growth. Because rhinos are so long-lived, even a declining population has a reasonable chance of surviving 85 to 170 years. Because no upper limit was put on population growth, some simulation populations grew to more than 100 individuals and thus became fairly immune to random processes.

Table 9. Results of simulation study of extinction in small populations of rhinos.

				85 years		170 years	
Juvenile mortality %	Adult mortality %	Number founders	R_0	% surviving	N	% surviving	N
13	5	10	1.35				
		20	1.35				
	6	10	1.12	90	34	82	168
		20	1.12	100	74	96	251
	7	10	0.94	68	16	48	50
		20	0.94	92	32	86	60
15	5	10	1.32	93	93		
		20	1.32	100	152		
	6	10	1.10	85	36	74	154
		20	1.10	98	61	96	259
16	5	10	1.30	95	86	94	711
		20	1.30	100	136		
	6	10	1.08	89	43	70	146
		20	1.08	98	62	95	215
	6.808	10	1.00	37	14	18	56
		20	1.00	72	19	52	41
		40	1.00	94	33	76	62
		80	1.00	100	64	98	97
20	5	10	1.24				
	6	10	1.03	87	34	67	100
	6	20	1.03	97	55	96	149

Adult mortality affects the growth rate and persistence of the populations more than does juvenile mortality; even slight increases in adult mortality have very large effects, while small increases in juvenile mortality have little effect.

Loss of genetic variability in black rhino reserves in Kenya

Soon, few black rhinos will exist outside of carefully managed and guarded parks and reserves. One consequence will be that formerly contiguous populations will be isolated and, unless animals are moved between reserves, inbreeding and loss of gene diversity within the populations could lead to their demise. (Because of the slow growth of rhino populations, even moderate inbreeding depression could cause populations to decline). I used a simulation program to examine the loss of genetic diversity from semi-isolated populations of black rhinos remaining on reserves in Kenya. The simulation program models the random transmission of genes through generations, given input parameters for population sizes, migration rates, population growth rates, limiting population sizes, and (though not shown here) mutation, and selection. Although the model assumes random mating within each population, population censuses can be (and were) adjusted to produce estimated genetically “effective population sizes”, Ne (the size of a randomly mating population that would lose genetic variability at the same rate as does the real population).

Eight Kenyan populations that have a reasonable probability of receiving sufficient protection from poaching were considered. Estimated population sizes and carrying capacities were obtained from reports by Peter Jenkins and others. it was assumed that only those rhinos within areas proposed to be fenced would be protected from poaching. The ratio of effective population size to census population size was perhaps optimistically assumed to be 1:2.

Simulations were run assuming that each population started growing from its 1985 numbers, with growth rates of 25%, 50%, 1290/a, 2160/a, or 270% per generation (1.3%, 2.4%, 50/a, 70/a,”or 8% per year). The first two growth rates match some of the more optimistic, but not unrealistic, growth rates obtained from demographic analyses. The latter three match estimates reported at the Cincinnati meeting for variability after populations reached carrying capacity, simulations were also run assuming that each population was begun at its carrying capacity. in all cases, random demographic fluctuations were incorporated into the population sizes, 170 years modelling the fluctuations that would be expected if births and deaths were independent (Poisson) processes.

Table 10. Population estimates used In analysis of gene diversity.

	1985		Carrying Capacity		
Park	census	Ne	Total	Fenced	Ne
Aberdare	80	30	600	100	50
Amboseli	15	7.5	150	50	25
Laikipia	60	30	50	50	25
Masai Mara	12	6	180	50	25
Meru	5	2.5	300	20	10
Nairobi	28	14	50	50	25
Nakuru	2(10)	5	80	80	40
Sollo	71	35.5	50	50	25

Notes: Although Nakuru had only 2 rhinos in 1985, It was assumed that more would be brought in, bringing the number used to start that population to perhaps 10. Based on reports of habitat degradation, it was assumed that the Solio population was currently above its long-term carrying capacity.

The genetic fates of the populations were monitored by the percent of the initial heterozygosity that would be expected to remain in each population, and by the overall gene diversity (the sum of within-population variability and between-population genetic variability) encompassed by the eight populations. The overall gene diversity can be thought of as the heterozygosity that would be present if all eight populations freely interbred. Populations were followed through 20 simulated generations (about 340 years).

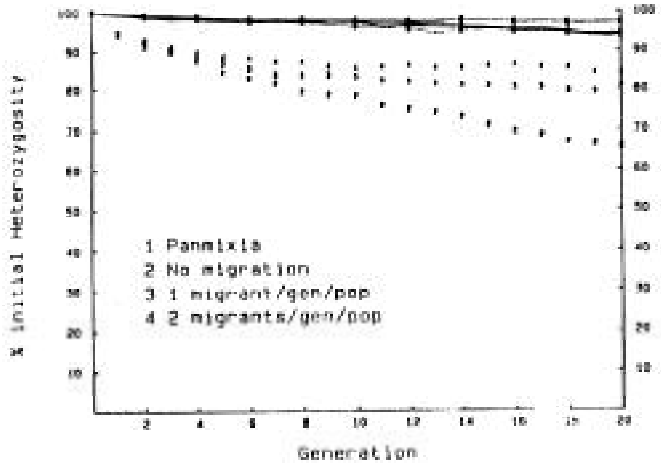
The fate of gene diversity over the twenty simulated generations is shown on the four accompanying figures, for either 25% or 50% population growth per generation, and either growth from 1985 levels to carrying capacities ("growing populations") or populations begun at carrying capacities ("stable populations"). In each case 25 simulations were run with no movement of animals between populations, the movement of one animal per generation per population, the movement of two animals per generation, and the movement of so many animals that the populations were essentially panmictic. Data points connected by lines display average (across 25 runs) of the total gene diversity present across all 8 populations at each generation; data points not connected by lines are the average within-population heterozygosities.

Table 11 Summarizes the simulation results for the cases shown in the figures, and also simulated populations with higher rates of population increase (average of 25 simulations in each case).

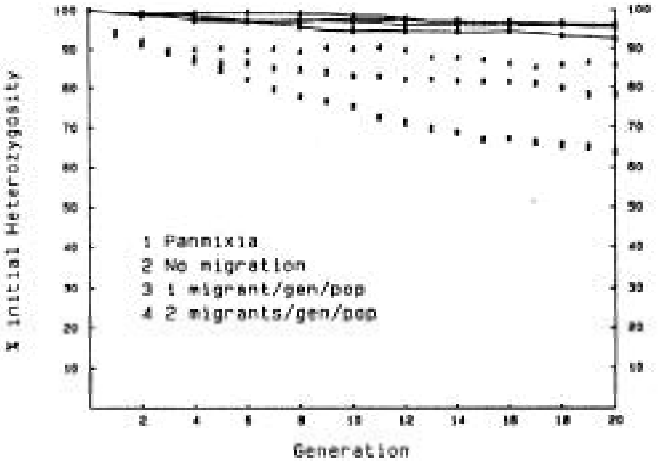
Over just 20 generations, more than 95% of the gene diversity would be expected to remain somewhere In the 8 rhino populations, assuming of course that all grow at the rates modelled and then hover around the assumed carrying capacities. Total gene diversity is preserved somewhat better If the 8 populations are kept fully isolated (""no migration" case), because different genetic variants can become "'fixed"

Figures 4-7. Results of simulation Study of decline of heterozygosity in small populations of Kenya rhinos. These graphs correspond to data in Table 11. Data points connected by lines represent average (of 25 runs) of the total gene diversity across all 8 populations of each generation. Data points not connected by lines are the average within-population heterozygosities. The graphs differ in the growth rate of the population per generation (25% or 50%) and whether the populations commence at 1985 levels (growing populations) or at the ultimate carrying capacity estimated for the reserve (stable populations).

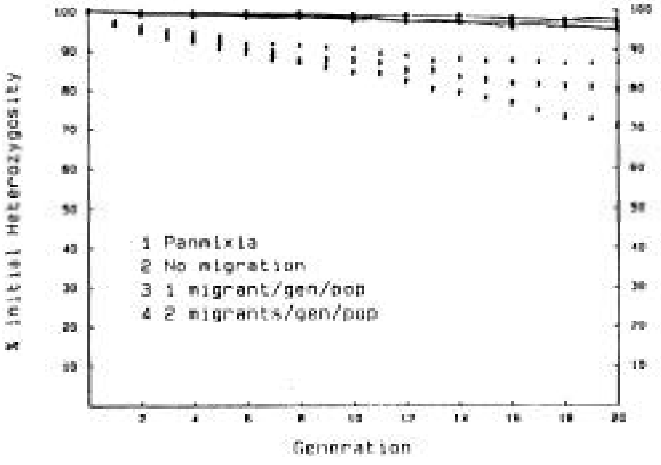
Kenya Black Rhinos: Growing Populations
INITIAL SUBPOP SIZES: 30 7 30 6 3 14 5 35
CARRYING CAPACITIES: 50 25 25 25 10 25 40 25
GROWTH RATE = 0.250



Kenya Black Rhinos: Growing Populations
INITIAL SUBPOP SIZES: 30 7 30 6 3 14 5 35
CARRYING CAPACITIES: 50 25 25 25 10 25 40 25
GROWTH RATE = 0.500



Kenya Black Rhinos: Stable Population
INITIAL SUBPOP SIZES: 50 25 25 25 10 25 40 25
CARRYING CAPACITIES: 50 25 25 25 10 25 40 25
GROWTH RATE = 0.250



Kenya Black Rhinos: Stable Population
INITIAL SUBPOP SIZES: 50 25 25 25 10 25 40 25
CARRYING CAPACITIES: 50 25 25 25 10 25 40 25
GROWTH RATE = 0.500

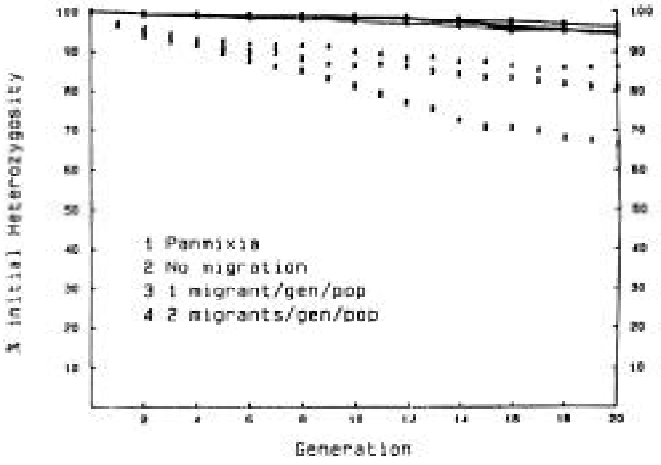


Table 11. Heterozygosity remaining at generation 200 as % of initial heterozygosity.

Annual growth	Generation growth	No migration	1 migrant	2 migrants	Panmixia
1.3%	25%	97	95	93	94
		66	81	84	94
2.4%	50%	96	92	95	96
	64	78	86	96	
5.0%	129%	93	95	95	97
	63	81	87	97	
7.0%	216%	95	94	95	96
	69	80	87	96	
8.0%	270%	97	98	92	96
	64	80	84	96	

Top values of each pair are average total gene diversity, bottom values are average within-population heterozygosities. Differences of less than 5% are probably not significant.

In each population, but the difference (in gene diversity preserved) between isolated populations, populations exchanging some migrants, and even a panmictic population is trivial for the rhinos.

Although total gene diversity is well maintained under all of the assumed population structures, heterozygosity is lost from within populations (i.e. some “inbreeding” occurs within each population). In the worst case (no migration), up to 35% of the heterozygosity would be lost, on average, from each isolated population. The average results from a much greater loss in the smaller populations (the Meru population would be expected to lose 64% of its heterozygosity ln 20 generations, even if it were begun at its carrying capacity of 20) countered”by lesser losses in the larger populations (Aberdare would lose about 180/s of its heterozygosity in 20 generations). As very rough rules-of-thumb, the effect (“‘inbreeding depression”) of a loss of less than 5% heterozygosity in any one generation is generally hard to detect, and animal breeders notice little or no effect of the loss of 1 % heterozygosity per generation continued over many generations. Thus, the small rhino reserves are probably too small to sustain populations for many generations, ln the absence of occasional inter-reserve movements of animals, free from genetic problems. Relatively low rates of migration, 1 or 2 migrants per generation per population, would probably be sufficient to prevent genetic problems. (This assumes migrants are as successful as are residents at breeding).

Neither starting the populations at carrying capacity (rather than 1985 levels) nor varying the population growth rate had much effect on the genetic results. This is because only rapidly growing populations were considered.. At even the lowest population growth, 25% per generation, most of the populations would reach carrying capacities in just a few generations. The genetic fates of these populations are much more determined by their limited sizes than by the number of founders.

General comments

Rhinos, both in the wild and in captivity, are probably not in Immediate danger of genetic problems arising from loss of diversity. Given the long generation time, all except the very smallest captive and wild stocks would experience minimal inbreeding in the next century or so. (For example, a population of 64 could be propagated for 6 generations with no matings between even distantly related animals). This optimistic genetic picture assumes, however, that protected rhino populations are currently at minima (i.e. they are at the worst phase of the population bottlenecks) and that they grow at reasonable rates over the next century.

Demographically, both wild and captive populations may be in serious trouble. The captive record is not good: as many as half of the animals have never reproduced, and birth rates approximately equal death rates. The large, and seemingly stable, captive population results in large part from the many wild-caught animals, not from a good record of captive breeding. As discussed in Cincinnati, there is reason to hope that this picture is changing, but the zoo community cannot yet claim to be able to sustain continuously growing stocks of black and white rhinos.

The small rhino reserves that are likely to receive adequate protection from poaching may not be large enough to prevent extinction due to random fluctuations in births and deaths, even under the most optimistic scenarios of environmental. and demographic constancy. The primary cause for hope for the African rhinos lies in the very long generation times and low-adult mortality (in the absence of poaching): traits that make population decline a very slow process, but also make rapid recovery difficult (witness the condor).

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DEVELOPING STRATEGIES FOR NORTHERN WHITE RHINOS

Session Chairman DAVID JONES

NORTHERN WHITE RHINOS IN GARAMBA NATIONAL PARK

Summary of presentation by Kes Hillman-Smith

Background

Garamba National Park in northern Zaire is now the last known place where the northern sub-species of white rhinoceros *(Ceratotherium simum cottoni)* exists in the wild with any chance of survival. At the turn of the century, the subspecies occurred from southern Chad, through South Sudan as far east as the Nile, and through the northern edge of Zaire to West Nile Province in Uganda (Hillman—Smith *et* al., 1986).

When the Park was established ln 1938 there were probably not more than 100 white rhinos there (Curry-Lindahl, 1972). Black rhinos *(Diceros bicornis)* have never occurred in this part of Zaire. The rhino numbers increased, until by 1963 there

was estimated to be between 1 000 and 1 300 rhinos (Park reports in Curry-Lindahi, 1972). Then, during the "Simba" rebellion, the Park was occupied by guerillas and poaching from Sudan was rife Curry-Lindahi states that approximately 100 was a "rough and perhaps optimistic estimate" in 1966. In 1969, control of the Park was regained and it became legal to shoot poachers not responding to orders to surrender. Rhino numbers rose again, and an aerial census in 1976 estimated 490+/—270 in the Park (Savidge *et al.,* 1976). After the end of an FAO aid project, funds from the directorate of the wildlife department (now called the institut Zairois pour Ia Conservation do la Nature, IZCN), for salaries and for maintenance and running of vehicles, were limited and usually late in coming. Park staff were not only unable to control poaching but many were involved in it to support themselves. in 1980, when we visited the Park as part of a pan-African rhino survey for IUCN and the New York Zoological Society, a project was proposed with the primary aim of conserving the rhinos. In 1983, on the basis of an intensive aerial survey, we estimated that there were between 13 and 20 rhinos remaining In the Park. In March 1984, the IUCN Garamba Rehabilitation Project started, funded by the World Wildlife Fund for Nature (WWF), Frankfurt Zoological Society (FZS) and the United Nations Educational, Scientific and Cultural Organisation (UNESCO). Some funds were also obtained from the Kenya Rhino Action Group (KRAG) and the Fauna and Flora Preservation Society (FFPS) specifically for ancillary work to assess the status of and to monitor the rhino population. Later some support was given by the Wildlife Conservation Fund. The results reported here are based on that and a part-time continuation of that work, in conjunction with the Garamba Rehabilitation Project and the IZCN biologist, Mr. Mankoto and his short term replacement Dr. Mbieme.

This report summarizes observations made during the period April 1 1984 ——October 1986.

Habitat

The Park covers 4 900 sq. km in the ""Guinea savanna belt In the north-east of Zaire, bordering on southern Sudan. The mean annual southern rainfall is 1 500 mm, which falls mainly in a long wet season from April to November or December. The southern two-thirds of the Park are largely open long grass savanna, dominated by *Loudetia arundinacea* and various *Hyparrhenia* species, which reach 2-3 m in height, with patches of certain species growing even taller. Sparsely scattered throughout the grassland are mature trees, predominantly *Kigelia africana* and *Vitex doniana,* with some patches of *Crossopteryx febrifuga* on shallow soils. Towards the edges and in the north are medium to sparse denisty areas of the deciduous bush woodland that dominates the surrounding country, comprising particularly, *Combretum collinum, Nauclia letifolia, Crossopteryx febrifuga, Hymencardia acida* and *Piliostigma thonningii.* The grassland is richly dissected by watercourses, with flowing water, marshes and patches of relict gallery forest. In the far north, the Park rises in more wooded, broken ground and scattered inselbergs to the Zaire/Nile watershed. The Park is far more open than the surrounding country and, according to reports, has been for some time, probably before its gazetting. Human factors, of clearing and burning, and elephants are probably the principal causes. About 90% **of** the grassland is burnt each year and there is little evidence of regeneration of woody vegetation.

Poaching is now largely controlled in the southern third of the Park. It still exists in the north but is not as severe as in many countries and meat is one of the main motivations. The rhino have probably always been more densely distributed in the south, but now they are confined almost entirely to the central part of the southern section, as far from the penetration of the poachers as possible.

Methods

Recognition of individuals has been the main means of emunerating and monitoring the rhinos end their social and population dynamics. They are found by a combination of ground and aerial work, since they are sparsely distributed. in the first few months, it was predominantly ground work, on foot in the Park. Effective ground work is, however, limited to the dry and early wet seasons, from January to May or June, after which the grass is too long. Now, due to other committments and lack of funds, the rhino monitoring is part-time and predominantly aerial, although reports are also made by the guards patrolling on foot in the rhino areas.

Horn configurations and ear and tail marks are the main characteristics used for recognition, although apart from one female with a large chunk missing from an ear and a young male missing half his tail, horn configurations are all that can be seen from the air. The most effective way to find them from the ground is with aerial support and ground-to-air contact. The most effective method of aerial monitoring has been found to be a series of flights over a few days, intensively searching as much of the area as practical, in blocks.

For each observation, the age, sex and identification of the individual is recorded as far as possible, together with the location, habitat, activity and any other notes. On the ground, activity is recorded over a period of time and notes made on behaviour and feeding. All spoor found are measured and locations of these, defecation sites, old skulls and any other marks are recorded.

Age classification is based on the criteria outlined In Hillman-Smith *et al.* (1986). Sub-adults are classified as being those animals three years or older, whether with their mothers or not and those who on the basis of horn and body size and behaviour appear to be less than adults. For the purpose of this analysis, infants and juveniles have been classified together as juveniles.

Numbers

We know that at least 18 individuals exist, or existed very recently, in the Park. Individual distinction is not always 100% certain from the air, nor is it always possible to sex an individual in quick aerial passes if the grass is very long or the animals do not display themselves well. This accounts for the fact that we are still not precisely sure how many adults there are, because some of these records may be re-sightings of the same animal appearing different under different conditions. We do know that in a recent series of recce flights we saw at least 17 different individuals, possibly 18, though we cannot confirm one pair of observations that could have been the same individuals at different times. Shortly after that series, the most recent calf was born, bringing the number to a minimum of 18. individual characteristics, however, indicate there are possibly more. Since April this year, we do not have confirmed observations of either of the sub-adults that left their dams when the next calf was born. This could indicate that something has happened to them, but more likely that they have dispersed out of the area we frequently search. it is not uncommon for any given animal to be unrecorded for some time and then to be seen again. This may be due to simply missing it or to temporary dispersals. The returns per unit effort in searching the most peripheral areas are so low, that in practice monitoring flights are mainly carried out over the central 5-6 000 sq. km.

Population Dynamics

The sex ratio of the confirmed sexed and known animals is 9 males to 8 females, excluding one calf and other possible adults. The age ratio of 22% male adults, 28% sub-adults and 22% juveniles is similar to that found by Owen-Smith (1973) for southern white rhinos (C.s.*simum),* although in his population the proportion of sub-adults was slightly higher. Unfortunately, in this population the majority of sub-adults appear to be males.

Reproduction has been good throughout the period of observation. Starting annual periods from the time of the March 1983. census, three calves were born and survived in 1983/4, one in *1984/5,* another three in 1985/6 and one more so far in this period. Two of the females have had an approximately two year inter-calf interval. All the confirmed known females at present have a juvenile with them, although one juvenile is around three years old and currently classified as a sub-adult.

We have no evidence of deaths throughout the effective period of the project. Mankoto and the guards reported one dead rhino in 1983 before the project, and a poacher was caught in 1984, before the project had vehicles and had become effective, who claimed to have killed two rhinos that year and two the year before. Two elephant carcasses that were found in the southern section on monitoring flights both had their tusks and the animals appeared to have died from "natural causes".

The sample size is too small for a statistical analysis, but during the operation of the project the mean population increase appears to have been greater than 10% per annum.

Range

The overall area in which we have observed rhinos is 676 sq. km, although guards have reported spoor sightings further afield than that. Observed ranges of individuals to date vary from 57 to 259 sq. km, with the females tending to have smaller ranges. The ranges are, however, of the order of 10 times greater than those recorded for southern white rhinos (Owen-Smith, 1973). This may be due to the low density, but there appear to be factors (one of which is probably the burning) that lead to changes in ranges. One female and two males, for example, were commonly found north of the Garamba River last year, but now are usually south of it. The move coincided with a time when burning had been particularly severe to the north, while some areas had deliberately been left unburnt to the south. For the one female, F4, the two parts of her range coincided with two different calves, but this has not occurred with other animals.

There is a central core area, where the chances of finding rhinos at any one time are greater than elsewhere. There are some indicators of territoriality among dominant bulls who nevertheless tolerate sub-adult or subordinate bulls, as was found by Owen-Smith (1973). This was not found by van Gyseghem (1979) on northern whites in Uganda, but his population was too small. Defecation and marking sites do not remain long or accumulate in this high rainfall, high termite-density environment, but the ranges of some of the older males remain peripheral to the central area, which is occupied primarily by one apparently dominant bull. We have also observed the aftermath of a fight between two bulls, who were unusually close to the core area.

Social Groups

Social relations are also similar to those found among southern white rhinos. 48% of observations were of groups of 2, the majority of which were mothers and calves. 35% of observations were of lone animals, the majority of which were adult males. Sub-adults are most commonly found with other animals, usually other sub-adults or females.

Larger, temporary amalgamations are only occasionally found, but it is not uncommon to find a number of groups, particularly those of females, remaining In the vicinity of each other and changing locations more or less together.

Discussion of future status

This is a very small population and as such is precarious, but observations during the period of the project are very encouraging. Reproductive success has been good, and while the project exists the status of the rhinos appear to be stable. The IZCN is now on a better footing with the new President Delegue General, Mankoto ma Mbaelele. Salaries have significantly increased and arrive in better time. However, if international support were to end at this stage the situation would deteriorate again to the detriment of the rhinos and this whole World Heritage Site. We therefore feel it is important that the Rehabilitation Project continues in at least some form at the end of its initial three years.

Further to the existing efforts, I have proposed the need for an assistant who could be based out in the field full-time, studying and monitoring the rhinos and the ecosystem, working with the guards who patrol the area and with an IZCN counterpart, in conjunction with the author's continued part-time work and aerial support. The proposal has been approved by IZCN but funds are needed. A long term continuation of the research in various forms would also be valuable for monitoring the situation and maintaining an international link without having to rely on the organisations that fund the Rehabilitation Project.

In the long term, the development of tourism would increase the national and local value of the Park and enable some form of self-support. This is not easy to sustain in such a remote area but it would be possible to develop specialist, but inevitably expensive tourism, beyond the limited local tourism that already operates. The existence at the Park of the only African elephant domestication centre is a major attraction and it is hoped that future funds could therefore be invested to re-develop the elephant school and the tourist facilities as well as to help to maintain the Park and the anti-poaching activities.

If the rhinos could be adequately protected, I estimate, on the basis of the current population structure, an inter-calf interval of 2-3 years and a projected loss of one animal a year. The doubling time of the population would be in the region of ten years. It is Therefore vital that this wild population is backed up by improving breeding of the captive population, which currently numbers 11 animals. If the various techniques for improving reproduction could be developed and successfully applied to the captive rhinos, it might be possible to envisage a future link in the management of the captive and wild groups to improve the status of both, by genetic exchange and re-introductions, It could be an exciting example of complementary action to save a sub-species from extinction and as a result to help conserve a valuable ecosystem and National Park.

Table 12. Some results from observations of northern white rhinos ln Garamba National Park, April 1984—October 1986.

A. AGE AND SEX RATIOS

Age ratio of confirmed known animals		
MA	4	22%
FA	5	28 %
S	5	28 %
J	4	22%

B. OBSERVED HOME RANGES

Individual	Size (sq. km)	Dates of observation
M2	185	Mar 84 - Oct 86
M3	112	May 84 - Oct 86
M4	259	Aug 84 - Oct 86
M5	105	Apr 85 - Oct 86
M6	218	Mar 86 - Oct 86
M7	174	Feb 86 - Sep 86
M8	86	Apr 86 - Sep 86
M9	132	Mar 86 - Oct 86
F1 and 1a	138	Apr 84 - Oct 86
F3, 3a and 3b	137	Apr 84 - Oct 86
F4, 4a and 4b	196	Jan 85 - Oct 86
F4 and 4a	82	Jan 85 - Apr 86
F4 and 4b	65	Aug 85 - Oct 86
F5 and 5a	93	Apr 84 - Oct 86
F6 and 6a	57	Mar 86 - Oct 86
3a/4a	90	Jul 85 - Oct 86

Mean range for adult males	183 sq. km(well known only)
Mean range for adult females	124 sq. km
Mean range for S2	143 sq. km
Range for S1	90 sq. km
Total range of direct Observations	676 sq. km

C. FREQUENCY OF OBSERVED SOCIAL GROUPS

Group composition	No. Observations	% of total
MA	103	32
FA	6	2
MA+FA	14	4
AU	9	3
MA+FA+S	11	3
MA + FA/s + J/s	27	8
MA + FA + S +J	3	0.9
MA + S/s	3	0.9
FA + S	8	2
FA + J	115	35
FAs + Js	1	0.3
FA + S + J	9	3
FAs + Ss + Js	1	0.3
S	5	2
S	18	6

M = male; F = female; U = unknown; A = adult; S = sub-adult; J = juvenile.

GARAMBA NATIONAL PARK — MANAGEMENT

Information presented by Charles Mackie (Garamba Rehabilitation Project)

The rehabilitation of Garamba is an IUCN project in collaboration with the Zairois Institute for Conservation of Nature, funded by the World Wide Fund for Nature (WWF), the Frankfurt Zoological Society and UNESCO. The objectives of the project are:

— to re-equip the Park;
— to restore the infrastructure;
— to retrain staff to control poaching.

Efforts are directed at the conservation of the entire Garamba ecosystem (not specifically at rhino conservation).

By the end of its initial three-year period, the project will have cost US$600 000. Two expatriates are employed full-time to assist in the Park management.

Guards are constantly on patrol in the main rhino area, with other guards nearby at a radio base, in constant contact with the Park headquarters. There are 24 patrol posts around the periphery of the Park with 4-6 guards living under uncomfortable conditions in each.

A major constraint to the management of the Park is the dense grass growth, which severely restricts horizontal visibility for at least half the year, and makes patrolling difficult. Hence an aircraft is particularly valuable for surveillance work.

At present, it would not be sensible to attempt to translocate the Garamba rhinos elsewhere; this is against government policy, and the animals appear to be relatively secure, and breeding well. A long-term international commitment to Garamba is necessary if current levels of support are to be maintained until the rhino population has at least doubled; this will require an investment of about US$1 million, in addition to the US$0.6 million already spent. To support a field biologist to closely monitor the rhinos and study various biological and ecological aspects, the initial annual cost would be about US$42 000 with continuation costs of US$26 000. Generation of revenue through tourism could not be significant until the Park's tourist facilities are considerably Improved; if tourism does develop, a procedure exists whereby the funds could be returned directly to the Park.

NORTHERN WHITE RHINOS IN CAPTIVITY

Information presented by David Jones (Zoological Society of London), Ulysses Seal (IUCN Captive Breeding Specialist Group), and Oliver Ryder (Zoological Society of San Die go).

When Dr. Faust of Frankfurt Zoo carried out a survey of northern white rhinos in captivity he determined that there was an old animal at San Diego, another at London, and one at Antwerp which has since died. There were also animals of doubtful origin at Riyadh and a male at Khartoum. The largest captive group was (and still is) at Dvur Kralove in Czechoslovakia. At the invitation of the zoo managers at Dvur Kralove, D. Jones and U. Seal visited this zoo in February 1986. The Czechoslovakian authorities indicated a strong interest in developing a constructive breeding programme and have maintained close liaison with the Captive Breeding Specialist Group (CBSG). Some work has been done to facilitate the management system so that more females can become productive. As part of this plan, the male from London was sent to Dvur Kralove in the summer of 1986.

There are currently ten animals at Dvur Kralove including one of mixed sub-specific ancestry. The oldest t and dominant cow — which originally came from Britain (Knowsley) ——has had offspring sired by three different males, the first of which was a southern white. The hybrid from this latter mating was born in 1977, while the pure-bred northern white rhino calves were born in 1980 and 1983. This same female was in oestrus in the summer of 1986 and was sequestered with a northern white bull; she can be expected to reproduce in 1987. Owing to the technical difficulties of shifting animals, the other females are without bulls during oestrus periods, and none have reproduced. The chief constraint at Dvur Kralove is the extremely cold winter climate. The animals cannot safely be allowed out of their housing for about seven months of the year, hence much of the mixing has to be done in a very restricted space. The animals are separately boxed and there is a natural reluctance on the part of the managers to mix animals which have not been in direct contact for a week or two. There have also been problems in the rhinos' diet, about which recommendations have been made by the CBSG deputation.

Moving the animals to a warmer climate would be the most desirable option but may not be realistic In view of political constraints. Adopting a two-year time limit for Improved breeding at Dvur Kralove, prior to suggesting a major translocation, 1a probably the best approach. The potential breeding animals are approximately 15 years old so they should theoretically have up to 15 years additional reproductive life. in the meanwhile, the Dvur Kralove staff must be given maximum encouragement and assistance with their efforts to build up this rhino group.

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REPRODUCTIVE RESEARCH UPDATE

Session Chairman BETSY DRESSER

INTRODUCTION

To start off this session, I would like to present the ideal scenario — the ideal for rhinos using the reproductive technology that has been hinted at during these meetings. That is, to collect semen for artificial insemination or embryos for embryo transfer, or better yet to be able to freeze semen and embryos and to move these cells around the country or around the world. We would like to bank these cells for years, thus helping to maintain an effective population size. That is the ideal. But the reality is that semen has been collected from black and white rhinos, it has been frozen and thawed successfully but it has never been used successfully to produce any offspring; artificial insemination procedures have been attempted in these species but have not yet succeeded. Embryos have not been collected from any species of rhinos nor, of course, have they been frozen. So we have a way to go.

Research is in its infancy and much of it needs to be applied, particularly the artificial reproduction techniques. in most cases — supplementing behavioural studies ——the greatest effort has focused on endocrine evaluations of oestrus cycles, and essentially we are still at the stage of trying to reliably determine the oestrus cycle of the rhinos in our care.

HORMONAL EVALUATIONS OF RHINO OESTRUS CYCLES AND PREGNANCY

A presentation was made by Dr. Ed Ramsay, formerly of the Oklahoma City Zoo, and Lonnie Kasman, formerly of the San Diego Zoo who, in a joint effort with Dr. Bill Lasley (also formerly of the San Diego Zoo) worked on a cooperative project with 19 zoos in North America.

With the forming of the AAZPA Species Survival Plans, around 1982, these researchers attempted to develop some strategies and techniques that the managers of rhinos in captivity might be able to utilize to help improve the captive breeding of their animals. The strategy that was adopted was to look at urinary steroid hormones; what was hoped was to better understand the reproductive physiology of the rhino (particularly the black and Indian rhinos) both through the oestrus cycle and pregnancy. Since blood is difficult to get from the animal when not immobilized, the strategy that has some obvious advantages is urine collection. In addition to being safer to collect, urine is readily available ln vast quantities!

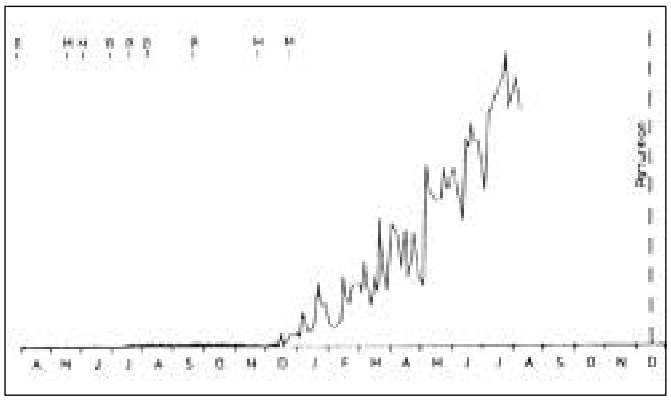
All of this work was done at the San Diego Zoo, and since the San Diego Zoo Endocrine Lab had a history of using radioimmunoassays for urinary steriod conjugate analysis, that is the method that was used. Preliminary studies there indicated that estrone sulphate, or estrone conjugates, would be useful for monitoring follicular activity in the Indian rhino and there was hope that it would also be useful in the black rhino.

Pregnanediol glucuronide (PdG) is an assay that was developed at the San Diego Zoo for monitoring luteal activity, or what was assumed to be luteal activity as a progesterone source in the rhino. That is the information that is presented in Figure 8.

The parturition which is indicated is the 1985 calf born at the Cincinnati Zoo. All the hormone values are indexed to creatinine to account for variability in the water content of the urine sample, so PdG is ng/mg creatinine.

Essentially what is seen are baseline levels (below the ensitivity of the assay) for about the first trimester of

Figure 8. PdG assay on one black rhino cow. B=breedings; 1 = mountings.



rpegnancy and then they begin to climb for approximately 12 months before parturition. The reason that the graph stops here is that Lonnie Kasman then left the San Diego Zoo and the assays ceased. This effort began in January 1983. At that time, there were only 27 black rhino females ln North America in the SSP programs. Of those, probably only about half were really considered to be potential breeders and then when the compliance factor was considered, there were actually only a few animals to study. However, this project shows the potential role that zoos can play in such research.

To further discuss urinary strategies, one thing that was hoped was to use the urine in the animals that consistently bred to diagnose pregnancy. There really has not been a good method in the past to monitor gestation or fetal viability. Then it was thought that it would be useful to look at the luteal phase, and ovarian function, in the case of pregnanediol luteal activity for comparison in the nonbreeding animal.

Unfortunately, pregnanediol was not found in the noncycling animal or the nonpregnant animal nor was it found in the first trimester of pregnancy in any of the black rhinos.

Figure 9 is a graphic representation of urinary pregnanediol ng/mg creatinine. The line on the right-hand side of the graph labelled zero indicates parturition during the course of the study and the bottom axis shows days prior to parturition. During the course of the study, eight animals that delivered were monitored and the graphed values represent samples from those eight animals. Unfortunately, the dots are not connected because frequently only half a dozen samples were received from an animal over the course of five or six months and to connect those dots would be deceiving. So this is merely a scattered representation of all the pregnancies that were looked at.

The one common factor is that around five months into gestation, between 12 and 9 months prior to parturition, a rise in pregnanediol was seen that continued throughout pregnancy in a rather consistent manner. So the serial sampling of three or four samples from an animal in late gestation can show measurable amounts of pregnanediol. It is believed that pregnancy in the animal can be determined. From data gathered during this project, it seems that the gestation range for black rhinos in captivity is 438-480 days, which is a range of 42 days with a mean of 463 days (a little more than *15* months). There is also an indication that post conception breeding occurs in these animals and can confuse rhino managers.

Figure 10 represents data obtained from Lottie from the Oklahoma City Zoo during a pregnancy. The scale on the left hand side is estrone sulfate or estrone conjugate and the scale on the right is pregnanediol glucuronide. The levels rise just into the measurable range (which ln this assay was about 8 ng/mg creatinine) about one year prior to parturition. The other thing that this figure shows is that there is a very precipitous drop-off in pregnanediol prior to parturition.

The project initially involved the black and Indian rhinos. in the second year, however, a few urine samples from two noncycling white rhinos were included in the assays. Non–cycling means that these are animals that were not being bred, were not showing any external signs of estrus and (as with the black rhino) had no measurable pregnanediol. Samples from two pregnant white rhinos were also collected, and levels of pregnanediol were found in late gestation that compared closely with those of the black rhinos.

In the Indian rhino it was possible to characterise both follicular and luteal phases and estrone conjugate and pregnanediol were found to be very useful for looking at both the estrus cycle and pregnancy. The Indian rhino is remarkably different to the black rhino, excreting a far higher level of steroids; during pregnancy the pregnanediol levels in the Indian rhino begin to climb at a similar time (about the beginning off the second trimester) but go up into the microgram/mi creatinine range.

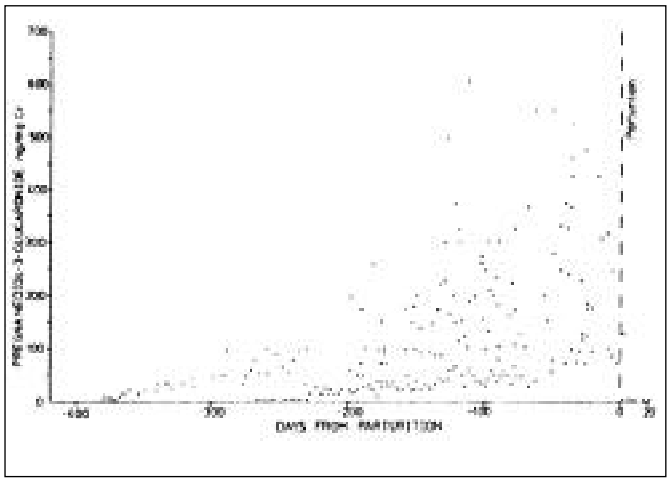


Figure 9. Urinary PdG mg/mg creatinine from 8 black rhino cows.

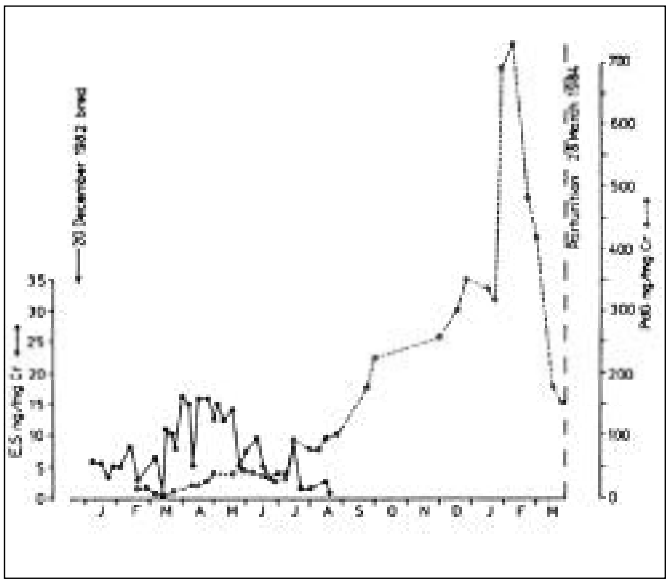


Figure 10. Estrone sulphate and PdG assays on one black rhino cow.

A cyclic pattern was evident ln estrogen values measured in the two Indian rhinos; at the end of a follicular episode, estrone sulfate levels dropped and then some 40 days later rose again, stayed up for 7-10 days and thereafter declined again. When estrogens declined, pregnanediol levels increased, indicating the production of a corpus luteum (which secretes progesterone). Some 14 days later the pregnanediol dropped again. These measurements tied in with behavioral indications of estrus ln these animals.

ESTRUS CYCLE DETERMINATION FROM CONDITION OF REPRODUCTIVE TRACT

Dr. Robert Wagner, veterinarian at the Pittsburgh Zoo. collected data from an 8 year old female southern white rhino over a period of 20 months. Attempts were made to collect samples biweekly. Behavioural observations for stage of estrus cycle were correlated with:

1. Rectal examination for uterine and cervical tone
2. Vaginal cytology
3. Urine hormones:
   (a) total estrogens (estradiol-17-B, estrone and estrone sulphate)
   (b) progesterone

Rectal examination of uterus and cervix revealed much Information about the female's cycle and anatomy. Ovaries were not palpable. The reproductive tract tone showed a change from being soft, pliable and flaccid to becoming firmer for some weeks prior to behavioural estrus, and then rapidly became well defined and turgid for two to four days during behavioural estrus.

Vaginal cytology was reported by Spellmore and Booth in AAZPA Regional Proceedings in 1981 for a black rhino. Similar findings were seen in the cytology of the white rhino. During diestrus, round non-cornified epithelial cells with distinct nuclei were seen, along with small quantities of mucus and debris. Then for about two or three days during proestrus the epithelial cells cornified and became angular ln shape with pyknotic and darker nuclei. A slight increase in mucus and debris was noted at this point. Also at this time the cells began karyolysis and lost their nuclei. A sudden change at estrus in the non-cornified to cornified cell ratio (NC/C) often occurred within 12 hours; commonly, greater than 70% of the cells became cornified with considerable debris noted. The epithelial cells of estrus were then irregular in shape with edges folded over, and contained no nuclei. The NC/C ratio would revert back within 12 hours to 50/SO or greater with cells resembling new diestrus cells. Rapidly changing cytology seen ln Pittsburgh's white rhino closely agrees with reports from San Diego Wild Animal Park of estrus lasting 15 hours based on behavioural observations (1985 SSP Survey).

Hormone analysis of urine for total estrogens and progesterones was completed as frequently as possible but occasionally time gaps of up to 12 days since collection would occur. Analysis was done by radioimmunoassay (RIA). Hormone concentrations were corrected for dilution by standardizing against creatinine levels. Baseline estrogen levels ranged from 200 to 900 pg/ml with small mid-cycle peaks of less than 900 pg/ml ranging between November and July. Total estrogens showed the best correlation with observable heat. Estrogen peaks of greater than 1 200 pg/ml occurred within four days of noted heat. From August to October multiple estrogenic peaks (less than 1 300 pg/ml) were seen with little pattern or regularity. During this time. poorly defined heats or no cyclic behavioural activity was seen. Progesterone peaks (0.12S-0.250 ng/ml) followed extremely close to declining estrogen peaks from November to July, then levels became erratic and poorly correlated. These hormone fluctuations may explain the lack of obvious estrus behaviour in Pittsburgh's female rhino from late summer to early winter. There seems to be a seasonal anestrus occurring in this female during this time.

From December to July, Pittsburgh's female has strong (easily observed) heats and regular estrus cycles. With approaching heat the uterus and cervix increase tone, the vaginal cytology changes from non-cornified to cornified cells and urine total estrogen levels peak. Behavioural estrus lasts three to five days. Progesterones rise after estrogen peaks and tone and vaginal cytology go back to baseline levels. Cycle length varies from 38 to 58 days with most cycles being 40 to 42 days. As mid-summer approaches, cyclic behaviour and observable heats are much harder to determine. This agrees with the non-cyclic activity in tract tone and cytology. Future goals are to isolate a LH-like compound in the urine, sonographic evaluation of ovaries for staging the cycle and eventually artificial insemination.

FURTHER RESEARCH ON METHODS FOR OVULATION AND PREGNANCY DETECTION

Dr. Richard Kock of the London Zoological Society presented results of studies done in collaboration with Dr. J.K. Hodges also of the London Zoo, on detection of ovulation and pregnancy in rhinos. The following is a summary of the results.

1. Comparison of urinary estrogen metaboiltes during pregnancy. Sequential hydrolysis of urine samples from mid-late pregnancy in the Indian, black and white rhino showed:
   (a) important species differences in the amounts and type of estrogen excreted;
   (b) large amounts of estrogens were detected during pregnancy in the Indian species. The most abundant estrogen component was estrone sulfate;
   (C) very low levels of estrogen were excreted in urine during pregnancy ln the black and white rhinos. Of those measured estradiol glucuronide appeared to predominate;
   (d) measurement of urinary estrogens may be useful for monitoring pregnancy in the Indian rhino but not at present in the other two species;
   (e) more studies are needed in the black and white rhinos to examine the presence of other urinary estrogens and to determine whether there is a preferential route of fecal excretion.
2. Measurement of urinary progesterone metabolites during pregnancy.
   Urinary pregnanediol-3x-glucuronide was measured during mid-late pregnancy in the Indian, black and white rhinos. The results showed:
   (a) elevated levels of PdG in all three species;
   (b) levels ln the Indian rhino were between 5-10 ug/mg creatinine whereas levels in the black and white rhinos were much lower at comparable stages of pregnancy (0.4-0.8 ug/mg Cr and 0.05-0.1 ug/mg Cr, respectively);
   (c) levels of PdG in all three species fell markedly (greater than ten-fold) within one week of termination of pregnancy (birth or abortion);

(d) measurement of urinary PdG appears to provide a useful method for detecting mid-late pregnancy in rhinos. Further work is needed to establish tests for early pregnancy.

3. Monitoring of estrus cycles and ovulation.
   In contrast to the Indian rhino, attempts to monitor the estrus cycle in black and white rhinos by measurement of urinary estrogen metaboiltes and pregnanediol-3x-glucuronide have so far proved unsuccessful. Other methods need to be investigated.
4. New assay methodology.
   A new, simple microtitre plate ELISA (enzyme assay) for urinary pregnanediol-3x-glucuronide has been developed and validated for all three species of rhino.

UPDATE ON DEVELOPMENT AND APPLICATION OF REPRODUCTIVE TECHNOLOGY TO RHINOS

As head of the research team of the Cincinnati Wildlife Research Federation (CWRF) which is a combined effort of the Cincinnati Zoo, Kings island Wild Animal Habitat and the University of Cincinnati College of Medicine, Dr. Betsy Dresser reported on the development and application of reproductive technologies to rhinos. At the Cincinnati Zoo , there are two breeding pairs of black rhinos and they have produced 13 offspring to date. At Kings Island, there is a group of white rhinos, and four nonpregnant cows that are being worked with now in some areas of embryo transfer technology.

It is the hope of the CWRF to eventually be able to do embryo transfer within the white and black rhino species and also at some point attempt interspecies embryo transfer between the black and white rhinos. There is a lot of talk about embryo transfer but until actual manipulation of these animals is tried, working with them is a little more difficult than is first thought. So, first there is a need to determine if catheters can be physically inserted into the cervix and uterus manipulated before superovulation by hormones can be attempted.

As has been mentioned by other investigators, there is a need to be able to determine the estrus cycle in rhinos, It will be important to know when we can artificially inseminate on when to breed these animals before embryo transfer can be attempted. And then, after that, we have to know when embryos can be recovered. Also, embryo recipients will need to be hormonally prepared in order to establish a pregnancy. At Kings Island in Ohio, in an ongoing effort to develop embryo transfer technology for white rhinos, animals were first immobilized, placed in sternal recumbancy and rectally palpated to evaluate the reproductive tract. To date, it has been determined that uterus and ovaries could be palpated. but it is often very difficult. Ultrasound equipment is now being used to aid these efforts.

Specula are being developed in order to visualize the cervix for catheter insertion. A lengthy catheter has been developed for this procedure and attempts to flush the uterus with fluids are underway. Once superovulation techniques are pursued. embryo recovery techniques will be correlated.

Another technique that the CWRF team have been trying with rhinos came out of work that is being done with domestic cattle. It involves a small radio transmitter that sends out pulses. It has been used successfully in cattle to determine internal body temperature. It is inserted into the vagina and is similar to the method used to measure internal body temperature in women when they ovulate and there is a measurable increase in body temperature. When a cow's internal body temperature increases, these pulses increase and are received through a radio receiver. Dr. David Zartman, of Ohio State University. has inserted many of these into cattle. He custom-made the transmitter for the rhinos (larger that that used in a horse). The rhino cows have been monitored for at least six months and a trend does appear to be emerging.

Dr. Terry Blasdel. research coordinator for the Houston Zoo, has organized a program to produce offspring from white rhinos at the Houston Zoo by artificial Insemination. This project involves at least eight other zoos in North America, but had not yet begun at the time of the meeting.

Session Chairman ERIC MILLER

HEMOLYTIC ANEMIA IN THE BLACK RHINO

Summary of presentation by R. Eric Miller (St. Louis Zoological Park), co-authored by Hugh Chap//n (Washington University School of Medicine), Donald E. Paglia (University of Cal/torn/a at Los Angeles) and Will/am J. Boever (St. Louis Zoological Park).

Hemolytic anemia in the black rhino *(Diceros bicornis)* is a frequent occurrence and cause of death in the captive population of this species. Twenty-eight episodes of hemolytic anemia have been identified in 21 animals in zoos in North America, Europe and Japan. Eighty percent of the affected rhinoceroses have died during their initial or a recurrent episode of the anemia.

In man and in domestic animals, hemolytic anemia may result from a variety of factors that lead to a decrease in the survival time of the red blood cells (RBC's) and their early intra- or extra-vascular destruction within the body. Intravascular destruction of the RBC's leads to the release of their hemoglobin into the serum (hemoglobinemia) and may result in its passage into the urine (hemoglobinuria). The latter results in a clear, dark red coloration in the urine that is often the first sign that a black rhinoceros is developing a hemolytic crisis.

The case that occurred in St. Louis in 1981 (studbook 183/STL 6) was typical of the majority of the cases (8). A nine-year-old nulliparious female was noted to be weak and passing red urine. She was anesthetized for further evaluation, and blood values reflected a marked anemia — a haemocrit of 14.5% (normally 45-50%) (6). in other cases this value has ranged from 4.5% to 36**%** on initial presentation. Nucleated red blood cells — cells that in the horse are indicative of intensive efforts to replace the RBC loss—— were noted Similar findings, including regenerative bone marrow, have been found in two subsequent cases. The St. Louis animal died during attempts to reverse the anesthetic, no doubt complicated by the severe anemia present. Necropsy find-

ings were unremarkable except for massive deposition of iron in the liver (3 000 ppm) and the digestive tract. Similar iron deposition had been noted in previous cases, and in one animal without any signs of hemolysis. Further evaluation is warranted to determine if this reflects a subacute or chronic stage to the peracute form of cell destruction that is the hallmark of the syndrome described here.

A common cause to link the majority of the cases of hemolytic anemia has not been identified. Leptospirosis is strongly suggested in several cases (1,4), including one recent case (Osaka 209/LAX 5). Two cases were noted in Frankfurt that temporarily responded to steroids (7). Fatal hemic parasitism has been noted in newly captured wild black rhinoceroses (9), but its relationship to hemolytic anemia is unclear (2). No evidence for similar parasitism in captive animals outside of Africa has been noted to date, and titers for *Ehrlichia* sp. and *Babesia* sp. using reagents for domestic animals have been negative. Attempts to identify the agents of equine infectious anemia, copper toxicity, equine arteritis and clostridial infection have not identified any of these as possible causes of anemia in the black rhinoceros.

In a previous survey (8), respondents reported that they had kept 98 black rhinoceroses in captivity from 1972 to 1982. Twenty-five deaths occurred in animals greater than one year of age, and 11 of these deaths were associated with hemolytic anemia. Additional animals were located, bringing the total number of episodes to 28 ln 21 individuals. No sex ratio or seasonality is apparent. The greatest difference in age at death was noted between wild-caught (average 13.6 years) and captive-bred animals (average 7.0 years). Familial groupings were evident in one vertical grouping (mother-daughter-grandaughter) at the Frankfurt Zoo and multiple siblings from pairs at St Louis (three of four) and Denver (two of three). However, these three families appear unrelated to each other and only account for eight of the 21 affected individuals. At Toronto and Memphis Zoos, two and three cases occurred at one- and ten-day time intervals, perhaps suggesting a common agent or exposure. (indeed leptospirosis was strongly suggested at Memphis). However, at the majority of institutions single deaths occurred with apparently normal black rhinoceroses in the same enclosure or nearby. Despite the pre-mortem exposure of one animal to isoniazid and two others with an inadvertent exposure to the rodenticide diaphacinon, no common environmental exposure could be found.

Further efforts to identify a cause for the syndrome were directed at finding a “common denominator”, a basic defect that could lead a number of factors, e.g. leptospirosis or a toxin exposure, to trigger a massive hemolytlc event. A”two–fold approach was chosen: (i) evaluate basic RBC parameters of stability and a possible immune basis for the anemia, and (ii) an evaluation of the function of the RBC’s via a study of their enzymes and metabolites. The former approach was designed to evaluate the stability of the black rhinoceros RBC and the apparent response of several European animals to steroids. The latter study was designed to evaluate the RBC enzymes of the black rhinoceros due to several similar hemolytic syndromes in some human populations that are due to specific enzyme defects in their RBC’s.

For the first study, specific Coomb’s reagents for the black rhinoceros were developed (3). Using black rhinoceros sera inoculated into rabbits, both anti-black rhinoceros whole sera and a more specific anti-IgG were developed. Reactions with these reagents have been negative in all presumed normal animals, and one of the two anemic black rhinoceroses studied to date. in the second individual in a hemolytic crisis, the test indicated a possible coating of the RBC’s with the C3 component of the complement system. in man, this may occur in a number of chronic conditions and does not necessarily indicate an immune, basis to the disease. The reagents continue to be available for use in any future cases of hemolytic anemia. To facilitate their use, they will most likely be disseminated to centers in North America, Europe and Africa.

Additional studies (3) also indicated an increase in osmotic fragility of the black rhinoceros RBC in saline solutions in comparison to man. The haemaglobin electrophoretic pattern of the black rhinoceros indicated two bands at a pH of 8.6, the majority (80%) of the hemoglobin migrating slightly distal to the region of the unstable human hemoglobin H. The significance of both findings remains uncertain at this time. Electrophoretic patterns of RBC membranes of affected and unaffected individuals found no discernible differences between the two.

A separate study (10) evaluated the red blood cell enzymes and metaboiltes of aerobic glycolysis, glutathione cycling, and nucleotide metabolism. Ten animals were tested’— seven of East African origin, including two during hemolytic episodes, and one who was the dam of three affected individuals; and three apparently normal animals from southern Africa. Though the values found differed markedly from human normals, no differences were noted between apparently normal and affected rhinoceroses. Values were comparable to those found in a previous study of two rhinos (5). Further tests on an anemic Individual found no differences between the time of the hemolytic crisis and the convalescent period, nor was evidence of a heterozygous carrier state evident in the dam of the affected animals.

An interesting notation to this study was the variation between the animals of the eastern and southern origin in two of the enzymes studied. The seven samples from the eastern animals had only one third of the 2,3-diphosphoglycerate activity, and twice as much reduced glutathione in their RBC’s as did the southern animals (10).

Another area of possible importance to the etiology of hemolytic anemia is the overall nutritional status of the captive black rhinoceros population. Nearly an exclusive browser in the wild, captive diets for this species often predominate in feeds more closely approximating those of a grazer. Four captive black rhinoceroses were assayed for alpha-tocopherol levels. Levels were undetectable in two, and levels 0.2 ug/ml and 0.23 ug/ml were found in two additional animals. Selenium levels were 0.122 ug/ml to 0.170 ug/ml in the four animals. Further vitamin and mineral evaluation of the captive and wild animals is planned. Assays from wild animals are needed to supply standard values for animals on natural feeds.

Suggested treatment for the syndrome at the present time remains empirical: (I) high doses of penicillin or tetracycline if the case is acute leptospirosis or other infectious agent; (ii) vitamin E and selenium supplementation due to their role in the stability of red blood cell membranes; and (iii) possible use of short-acting steroids due to the apparent response of several European cases.

In all future cases, major emphasis must be given to the re-evaluation of each of the possibilities discussed —leptospirosis equine infectious anemia, copper toxicity, clostridial infect ion, hemic parasitism, undetected infectious agent or exposure to a toxin. Wherever possible, frozen tissue and serum

should be saved and stored at -75 degrees C for future reference.

Possible future avenues of research identified at the meeting include: (I) repetition of many of the previous tests on additional black rhinoceroses and also on white and Indian rhinoceroses, (ii) further evaluation of the immunological status of these animals in addition to the continued use of the Coombs reagent, (iii) further evaluation of the stability of the black rhinoceros RBC and its hemoglobin, (iv) evaluation of the iron metabolism of this species and attempts to identify a possible chronic stage of the anemia process, and (v) an overall evaluation of the nutritional status of this species In captivity. One emphasis of the latter study should be the determination of vitamin E and selenium levels in both captive and wild populations. The importance of a multi–faceted diagnostic approach was emphasized in a species in which so little is known. in man, with a much broader data base available, the cause of less than 50% of nonspherocytic hemolytic anemia is identified.

Since the syndrome has not been reported in white and Indian rhinoceroses, results from these species may help to establish a comparative data base for the black rhinoceros. A blood collection protocol for diagnostic and genetic studies in black, white and Indian rhinoceroses has been distributed to North American and European institutions holding these species (copies are available on request from the senior author).

Finding the specific etiology for the hemolytic crisis so frequent in the captive black rhinoceros population rests on further research in the areas enumerated above and perhaps others yet to be identified. The authors welcome suggestions of additional tests and approaches to this perplexing problem in the successful maintenance of this species in captivity.

Authors' notes

(i) Collecting large volumes of blood from the black rhinoceros can be difficult if the ear vein is used as the primary venipuncture site. Animals at St. Louis have been routinely bled from a large vein that passes over the medial carpus and ante-brachium. Though it is not always visible under the thick skin, a tourniquet applied proximally on the leg allows it to be palpated and cannulated. Up to one litre of blood has been collected rapidly from this site.

(ii) Since the Cincinnati meeting, an additional three adult (14.16 and 24 years of age) black rhinoceroses have died of hemolytic anemia in North America. The deaths occurred from November 2 to December 17, 1986. Preliminary laboratory data from these cases parallels that from previous hemolytic events. Two of the cases were tested with the autoimmune reagents described in this paper, and both were negative. Further tests are pending. No common factors could be identified to link the cases.

HAEMATOLOGICAL STUDIES OF BLACK RHINOS IN ZIMBABWE

Summary of presentation by Raoul du ToIt (IUCN African Elephant and Rhino Specialist Group), co-authored by Beverley Paul (University of Zimbabwe)

Various haematological studies were carried out with blood samples from 31 black rhinos that were translocated from the Zambezi Valley, Zimbabwe, in mid-1986.

In a field laboratory, within 3 hours of the collection of each sample, the following procedures were carried out haematocrit, white blood count, red blood count measurement of haemoglobin, plasma protein, erythrocyte sedimentation rate, and osmotic fragility; preparation of slides for differential cell counts, reticulocyte counts and parasite screening Additional blood samples from each animal were transported too Harare on wet ice, where standard blood analyses were performed on a Coulter counter (within at most 48 hours, and generally within 24 hours, of collection) in Harare, additional tests were carried out to investigation haemoglobin stability: isopropanol precipitation, heat test acidified glycerol lysis time test, and staining for Heinz bodies with methyl violet. Human blood specimens stored for similar periods were used as controls. Haemoglobin electrophoresis was performed on cellulose acetate Giucose-6-phosphate-dehydrogenase was assayed using a commercial kit (Sigma), which had been supplied by St. Louis Zoo.

The findings of these investigations are to be published (Journal of Zoology, in press). Consistent results were obtained from the standard haematological tests, an measurements of haemoglobin, haematocrit, and cell count conform closely with those obtained by veterinarians at Whipanade Park, using blood from a few captive black rhinos Thus it is felt that these data constitute reliable baseline information on the haomatology of the species Reticulocytes, not generally seen in rhino blood smeared occurred in some of the samples. The osmotic fragility of the red cells was somewhat greater than that of human red cells with 50% lysis occurring at a salt concentration of about 4.9 g/l. A significant observation was that all samples showed rapid precipitation of haemoglobin when inclubated will isopropanol. Heinz bodies could be demonstrated by methyl violet staining in up to 10% of fresh red cells. Very high levels of G-6-P-D activity were found in the red cells.

These results, Indicating an inherent tendency towards collapse of haemoglobin under oxidant stress, are obvious highly relevant to the problem of intravascular haemolysis. It seems unlikely that there is any single agent responsible for triggering haemolysis episodes; these are probably the end result of a variety of oxidant stresses.

There are indications that some die-offs of black rhinos in the wild could be related to haemolytic anaemia (e.g. about 30 rhinos died in Tsavo National Park in 1960-61, due to what was tentatively described as "'nutritional anaemia"). With wild animals, it would be worth investigating if parasitaemia aggravated by Inadequate nutrition, capture stress and other debilitating factors, is associated with haemolytic anaemia-abnormal haemoglobin and red cell enzyme systems may have developed in rhino as an evolutionary response to parasitaemia (as with sickle cell anaemia and possibly G-6-P D deficiency in humans), but under extra physiological stresses the balance could tip towards excessive haemolysis The Zambezi rhinos, from which blood samples were taken. were translocated to another reserve in Zimbabwe, where at least 20% of them died some weeks after translocation. in the pathological examinations that were carried out on a couple of sick and dead rhinos in this group, an unidentified piroplasm parasite was found In blood smears to a greater extent than in biood smears taken at the time that the animals were first captured, and large amounts of haemosiderin were found in spleen and liver tissue. This indicates a possibility of the mortality being due to stress-induced parasitaemia and a degree of haemolytic anaemia (although it has also been suggested that the deaths were due to the use of the drug ivermectin, for controlling skin and gut parasites). Further

investigation of rhino blood parasites and haematology is Intended In the hope of clarifying these health problems before more animals are lost In translocation operations, which will become an increasingly important part of rhino conservation In Africa.

POPULATION AND VETERINARY STATUS OF BLACK RHINOS IN THE UNITED KINGDOM

Summary of presentation by Richard Kock
(Zoological Society of London)

Introduction

The black rhino population in the British isles numbers 12 at present: five wild-caught and seven captive-bred individuals. The latter derive from two genetic lines. One pair came direct from East Africa to the United Kingdom In 1950. The other genetic line is derived from a pair at Hannover which were wild-caught in 1955 and 1957, and two Whipsnade animals and one London animal which were also wild-caught. Fifteen animals In total were caught from the wild. Twenty-four individuals have been born in captivity since 1958. From 1969-1986, 24 deaths occurred including both captive-bred and wild-caught individuals. The major reason for this poor record includes a high mortality in both sexually Immature and mature individuals. A relatively short reproductive period over the life span and a long calving internal are also problematic. Deaths In this species, when compared to the white rhinoceros in captivity, are premature.

Of the 24 deaths recorded, 21 died between October and May, there was one still-birth in July and two deaths between May and October, but both of these had been Ill during the previous winter. In general the clinical syndromes recorded are associated with winter management, i.e. indoor housing, fluctuating climatic conditions, dry fodder nutrition and inactivity. There appears to be no sex or age susceptibility to Illness.

Nine collections have exhibited the black rhino, including 5 currently.

From 1969-1986, 20 deaths occurred in collections as follows:

Chester (5), Marwell (2), Bristol (6), Dublin (3), Paignton (1), Manchester (2), Whipsnade (2), London (1) and Howletts (2). The most "successful" records are from London/ Whipsnade and Howletts/Port Lympne. Only three post-natal deaths (two juveniles and one adult which was Ill on arrival from Bristol) occurred in these collections. Five offspring from these two zoos are at present alive In Great Britain. The two animals at Howletts are in their sixteenth year of captivity and include a captive born animal. The animals at London/Whipsnade are over 20 years old. A major difference between these two collections and the rest is in feeding management, with more browse and green foods being provided during the summer due to a rural location.

Clinical histories 1969-1986

From the case records available only a few individuals died without clinical signs prior to death. The clinical signs have included nasal discharges; with muco-purulent material, serosanguinous fluid and frequently whole blood clots. Skin ulcers, diffuse and punctate in appearance and with a remarkably regular patterning over the skin surface, were common. A few cases presented with diarrhoea, laminitis or haemoglobinurea. During periods of illness animals were in general lethargic and on occasions inappetant. Many of the animals have shown respiratory distress In the last two or three days of illness particularly where recumbency was evident.

Due to the difficulty of clinical examination without anaesthesia in this species clinicians rarely performed extensive diagnostics.

Haematology

The information available is primarily from the Zoological Society's collections (Table 13). It appears that the red cell numbers, haemoglobin concentrations, packed cell volumes and mean cell volumes are extremely variable in the individuals examined when compared with the white rhino. There appears to be no correlation between the time of year and the red cell/mean cell volume values or the presence of heinz bodies. The heinz body findings are unlikely to be significant as they are a common occurrence in white rhinos. High mean cell volumes have been recorded in several animals and this was suggested to be due to a vitamin B or folate deficiency. it may have been an indication of a response to red cell loss by haemolysis. In general, the black rhinoceros has lower red cell haemoglobin values, packed cell volumes and higher mean cell volumes than the while rhinoceros. The only comparative data to hand are from an individual case in another collection which showed dramatically lower red cell haemoglobin and packed cell volume values to those in the collection. it is worth noting here that none of-the deaths in the Society's collections have been during a haemolytic crisis, as has occurred in other collections in this country and abroad.

Table 13. Haematological data from African rhinos, obtained by the Zoological Society of London.

	Black rhino (n = 7)			White rhino (n = 16)		
	Lowest	Mean	Highest	Lowest	Mean	Highest
Red cell count (x 1012/1)	2.69	4.80	6.90	5.48	6.82	8.16
White cell count (x10^9/1)	3.0	8.5	14.0	4.7	8.6	12.5
HaemoglobIn (g/l)	9.76	14.70	19.64	13.94	17.03	20.13
Packed cell volume (%)	30.7	41.6	52.6	37.9	46.4	54.9
Mean cell volume (f1)	76.1	86.8	114.2	67.3	68.0	69.1
Mean cell HaemoglobIn (pg)	28.5	30.6	36.3	24.7	25.0	25.4
Erythrocyte Sedimentation rate (mm/hr)	2.00	22.50	54.00	5.00	16.98	33.00
Platelets (x 10^9/I)	14.3	314.2	614.1	2.28	5.34	8.41
Reticulocytes						
Neutrophils (x10^9/I)	0	0	0	0	0	0
Lymphocytes (x 10^9/I)	2.38	5.09	7.79	2.28	5.43	8.41
Monocytes (x 10^9/I)	0.00	0.24	0.95	0.00	0.32	0.83
Eosinophils (x10^9/I)	0.00	0.22	0.72	0.16	0.41	1.00

Biochemistry

The biochemical parameters did not show any consistent abnormality except for very low plasma vitamin E levels of less than 0.1 mu/ml. Low values are seen frequently in white rhinos and elephants so this is difficult to interpret. Plasma vitamin A levels varied between 15-140 iu/litre. Very little

biochemistry is available from animals terminally; one case showed raised creatine kinase and urea, a very high calcium/phosphorus ratio and hyperglobulinaemia.

Bacteriology, etc.

The bacteriology of oral and skin ulcers was inconsistent and bacteriology of post-mortem materials was inconclusive. In general virological investigations have not been performed. No evidence of fungal infections of any significance was recorded.

Pathology

Relatively few cases were investigated thoroughly at post-mortem. For example, only viscera was examined from certain individuals. Full histological series were rarely obtained with tissues taken according to the gross post-mortem.

Fortunately a few cases were thoroughly examined and provide valuable information. General comments on the table of pathological findings (Table 14) follow.

There appears to be a pattern of pathological change with similarities between individuals. The suggestion is that a number of animals suffered from a similar condition. This was not recognized hitherto due to the scattered nature of the cases and inconsistent examination through the involvement of many individual clinicians and pathologists.

A significant number of animals were found on histology, to have heavy haemosiderin deposition in a variety of tissues which, although not an uncommon finding in normal horses, is rarely seen to the extent in evidence in the rhinoceroses. This suggests haemolysis during life. These changes have also been noted in zoo equids to a lesser extent.

An interesting finding was glomerulopathy which requires further investigation. Fortunately a number of tissues are available for this purpose from previous cases. The possibility of immune complex deposition in the kidney is under investigation. The ulcerative dermatoses were frequently encountered but rarely investigated histologically. The range of findings in the skin suggest low grade dermatitis unlikely to be infectious in origin with hyperkeratosis, vesication, arterial changes (including endarteritis obliterans) and deposition of pigments amongst other pathological findings. Ulcerative and inflammatory changes of the alimentary tract were common. One case of a typical myopathy was reported, one liver hepatosis and two cases with pathology in the respiratory system other than emphysema. Two cases which died acutely with apparently no preliminary signs showed evidence of acute haemolysis, one with extensive alimentary tract ulceration and the other with apparent acidosis in the colon.

In summary, the pathogenesis of the ‘condition’ (if it is one condition) involves the development of ulcers in the skin and alimentary tract with mild inflammatory changes suggesting ischiemia rather than infectious agents and glomerulopathy, the cause of which is undetermined at present. in addition the deposition of pigments (predominantly haemosiderin) suggests haemolysis may be an important component of the syndrome.

Conclusion

In the opinion of the author there is sufficient evidence of a syndrome affecting black rhinos in captivity in the British Isles and leading to abnormal mortality in the species in captivity. The most likely predisposing factors or causes are winter nutrition and possibly stresses at this time of the year including enclosed housing, inactivity and fluctuating environmental temperature. The black rhino is almost exclusively a browser and the rations in captivity during the winter have been based on dry matter (primarily lucerne hays) and concentrated foods based on cereals. These diets are not consistent with the natural dietary intake and might lead to malnutrition in the species. The clinical response in a few cases to vitamin supplementation, particularly A and E, is

Table 14. Summary of pathological findings ——black rhinos———British Isles ——1969-1986

Local ID	Birthdate	Sex	Location	Skin Ulcers	Ulcers/ inflammation Alimentary Tract	Pigment Deposition Haemosiderin/ Lipofuschin or unidentified	Kidney Pathology (Giomerulopathy)
Willie	01.07.50	M	Bristol	*	*	*	*
Stephanie	01.07.50	F	Bristol	*	*	*	No histo
Rebecca	17.05.70	F	Bristol	*	*		
Rupert	28.06.65	M	Whipsnade	*	*	*	*
Kes		20.09.78	M	Marwell	*	*	* *
Joanna	15.11.72	F	Paignton	+	+		
Susie	01.07.56	M	Chester	*	*	*	*
Paul	01.07.63	F	London	*	*	*	+
Laura	01.07.60	M	Dublin?	+	+*	*	*
Johnny	01.07.65	F	Dublin	No histo			
M’kuzl	31.08.73	M	Whipsnade	No histo	+*	No histo	No histo
Linda	20.11.73	F	Chester	+			+ (myoglobin deposits)
Kijana	25.11. 70	M	DublinNo histology. Gross evidence of acute haemolysis and ulcers (skin & gut)				
Katie	16.09.79	F	MarwellAcute haemolysIs and colon acidosis. No histology.				

• Histology reported — positive findings
+ Gross findings
No histo: Histology not completed or reported

suggestive of a deficiency. These elements are liable to degradation in dry forage during the winter and may be a component of the syndrome. It is notable that the collections which have been most successful have provided large amounts of green food in the form of cut grass or browse during spring, summer and autumn.

Two facts are clear from this review. There needs to be an improved coordination between collections to ensure optimal investigation of cases and a more detailed examination of available tissues is necessary.

In the Society we have initiated a change in the nutritional management of the rhinos which includes an attempt at a browser ration for the black rhino and an improved supplementation of elements considered to be deficient in winter diets. The browser concentrate is fed at approximately 6 kg per day and the analysis is shown in Table 15. Lucerne hay is also provided plus vitamin E cubes to a level ensuring an intake of 6 000 international units per day per animal.

Table 15. Analyses of diets of black rhinos at London/ Whipsnade. All values are calculated to nominal 10% moisture content; all values are total calculated values; 1 mcg retinol = 3.3 i.u. vitamin A activity; total retinol content includes the retinol equivalent of carotene; 1 mcg B-carotene = 1.6 i.u. vitamin A activity: 1 mcg cholecalciferol = 40.0 i.u. vitamin D3 activity; 1 mg tocopherol = 1.1 i.u. vitamin E activity; 1 MJ = 239.23 calorIes.

General Purpose Diets

1. Bovine (Browser) Breeder Pellets
2. Bovine (Browser) Maintenance Pallets

		1	2			1	2
Crude Oil	%	4.5	2.8	Glycine	%	1.07	0.65
Crude Protein	%	16.4	12.9	Aspartic Acid	%	1.03	0.71
Crude Fibre	%	10.6	15.2	Glutamic Acid	%	2.98	2.36
Ash	%	10.9	10.7	Proline	%	1.04	0.88
N.F.E	%	47.6	48.4	Serine	%	0.66	0.47
				Hydroxyproline	%	–	—
Dig. Crude Oil	%	4.2	2.5	Hydroxylysine	%	—	—
Dig. Crude Protein	%	14.4	10.5	Alanine	%	0.12	0.12
Tot. Dietary Fibre	%	28.5	35.6				
Pectin	%	2.7	2.7	Calcium	%	1.20	1.24
Hemicellulose	%	13.2	14.2	Phosphorous	%	1.14	1.05
Cellulose	%	10.0	11.7	Phytate Phosphorous	%	0.33	0.30
Lignin	%	2.6	7.0	Sodium	%	0.73	0.72
Starches	%	16.8	16.4	Chlorine	%	1.11	1.09
Sugars	%	12.9	11.6	Magnesium	%	0.65	0.55
				Potassium	%	1.41	1.26
Gross Energy	MJ/kg	14.6	14.1				
Dig.. Energy	MJ/kg	9.4	7.7	Iron	mg/kg	194	153
Met. Energy	MJ/kg	8.5	6.9	Copper	mg/kg	18	16
				Manganese	mg/kg	117	116
Myristoleic Acid	%	–	—	Zinc	mg/kg	114	108
Palmitoleic Acid	%	0.05	0.02	Cobalt	mcg/kg	1104	1107
Oleic Acid	%	1.02	0.71	Iodine	mcg/kg	1431	1419
Linoleic Acid	%	1.19	0.58	Selenium	mcg/kg	356	314
Linolenic Acid	%	0.71	0.40	Fluorine	mg/kg	69	69
Arachidonic Acid	%	0.35	0.13				
Clupenodonic Acid	%	–	—	Retinol	mcg/kg	27889	26667
				Cholecalciferol	mcg/kg	103	52
Lauric Acid	%	0.06	0.03	dl %Tocopherol	mg/kg	116	69
Myristic Acid	%	0.20	0.15				
Palmitic Acid	%	0.44	0.33	Vitamin B1	mg/kg	13.8	9.5
Stearic Acid	%	0.13	0.07	Vitamin B2	mg/kg	10.3	6.3
				Vitamin B6	mg/kg	9.1	5.5
Arginine	%	1.10	0.76	Vitamin B12	mcg/kg	81.0	40.7
Lysine	%	0.80	0.54	Vitamin C	mg/kg	160.0	111.0
Methionine	%	0.22	0.16	Menadione	mg/kg	41.4	36.7
Cystine	%	0.24	0.18	Folic Acid	mg/kg	72.5	61.0
Tryptophan	%	0.24	0.18	Nicotinic Acid	mg/kg	45.1	31.6
Histdine	%	0.39	0.28	Pantothenic Acid	mg/kg	35.5	24.6
Threonine	%	0.60	0.42	Choline	mg/kg	1248.0	902.0
Isoleucine	%	0.66	0.46	Inositol	mg/kg	995.0	879.0
Leucine	%	1.13	0.79	Biotin	mcg/kg	519.0	378.0
Phenylalanine	%	0.71	0.50	p Aminobenzoic Acid	mg/kg	—	—
Valine	%	0.78	0.57				
Tyrosine	%	0.55	0.38	6 Carotene	mg/kg	49.6	50.2
Taurine	%	—	—	Carophyll Red	mg/kg	—	—

Vitamin E Supplementary Foods

1. Vitamin E Cubes
2. High Potency Vitamin E Pellets

		1	2			1	2
Crude Oil	%	6.4	16.8	Glycine	%	1.26	0.37
Crude Protein	%	17.4	9.3	Aspartic Acid	%	1.40	0.45
Crude Fibre	%	7.1	8.2	Glutamic Acid	%	2.76	1.50
Ash	%	9.1	15.6	Proline	%	0.91	0.61
N.F.E	%	50.0	40.1	Serine	%	0.72	0.29
				Hydroxyproline	%	0.02	—
Dig. Crude Oil	%	5.6	16.3	Hydroxylysine	%	—	—
Dig. Crude Protein	%	15.7	7.3	Alanine	%	—	0.09
Tot. Dietary Fibre	%	19.5	24.0				
Pectin	%	3.4	2.2	Calcium	%	0.85	0.34
Hemicellulose	%	8.5	12.6	Phosphorous	%	0.47	0.34
Cellulose	%	6.5	7.8	Phytate Phosphorous	%	0.23	0.20
Lignin	%	1.1	1.4	Sodium	%	0.08	0.14
Starches	%	31.0	9.2	Chlorine	%	0.15	0.24
Sugars	%	6.6	15.1	Magnesium	%	0.27	0.35
Gross Energy	MJ/kg	15.4	16.3	Potassium	%	1.03	0.96
Dig.. Energy	MJ/kg	11.5	11.8	Iron	mg/kg	145	61
Met. Energy	MJ/kg	10.4	10.6	Copper	mg/kg	10	7
Myristoleic Acid	%	0.04	—	Manganese	mg/kg	31	43
				Zinc	mg/kg	25	7
Palmitoleic Acid	%	0.22	0.08	Cobalt	mcg/kg	48	36
Oleic Acid	%	0.48	1.34	Iodine	mcg/kg	241	85
Linoleic Acid	%	0.66	2.18	Selenium	mcg/kg	120	163
Linolenic Acid	%	0.71	0.40	Fluorine	mg/kg	17	6
Arachidonic Acid	%	0.06	0.09				
Clupenodonic Acid	%	0.01	—	Retinol	mcg/kg	24799	24501
				Cholecalciferol	mcg/kg	10	—
Lauric Acid	%	0.01	0.09	dl %Tocopherol	mg/kg	23342	92651
Myristic Acid	%	0.07	0.25				
Palmitic Acid	%	0.25	0.49	Vitamin B1	mg/kg	4.0	4.0
Stearic Acid	%	0.10	0.20	Vitamin B2	mg/kg	4.1	2.0
				Vitamin B6	mg/kg	3.1	1.2
Arginine	%	1.25	0.47	Vitamin B12	mcg/kg	4.9	0.4
Lysine	%	0.71	0.34	Vitamin C	mg/kg	57.0	58.0
Methionine	%	0.32	0.12	Menadione	mg/kg	31.1	30.7
Cystine	%	0.29	0.12	Folic Acid	mg/kg	1.2	0.5
Tryptophan	%	0.25	0.13	Nicotinic Acid	mg/kg	45.1	31.6
Histdine	%	0.36	0.19	Pantothenic Acid	mg/kg	12.0	9.7
Threonine	%	0.62	0.27	Choline	mg/kg	1149.0	459.0
Isoleucine	%	0.67	0.28	Inositol	mg/kg	2349.0	509.0
Leucine	%	1.09	0.51	Biotin	mcg/kg	270.0	183.0
Phenylalanine	%	0.77	0.34	p Aminobenzoic Acid	mg/kg	—	—
Valine	%	0.84	0.38				
Tyrosine	%	0.51	0.25	6 Carotene	mg/kg	49.5	49.0
Taurine	%	—	—	Carophyll Red	mg/kg	—	—

Supplementary Foods

1. Rhino Cubes
2. Zebra Cubes

		1	2			1	2
Crude Oil	%	7.3	5.8	Glycine	%	0.41	1.48
Crude Protein	%	9.4	17.5	Aspartic Acid	%	0.64	1.25
Crude Fibre	%	18.7	11.8	Glutamic Acid	%	1.57	3.09
Ash	%	9.9	7.8	Proline	%	0.62	1.01
N.F.E	%	44.7	47.1	Serine	%	0.35	0.74
				Hydroxyproline	%	–	—
Dig. Crude Oil	%	6.9	5.4	Hydroxylysine	%	—	—
Dig. Crude Protein	%	7.5	15.6	Alanine	%	0.09	0.07
Tot. Dietary Fibre	%	43.9	30.2				
Pectin	%	2.4	3.2	Calcium	%	1.06	1.56
Hemicellulose	%	18.0	13.5	Phosphorous	%	0.53	0.66
Cellulose	%	18.1	11.0	Phytate Phosphorous	%	0.26	0.28
Lignin	%	5.4	2.5	Sodium	%	0.67	0.48
Starches	%	10.1	21.7	Chlorine	%	1.05	0.70
Sugars	%	9.4	7.0	Magnesium	%	1.20	0.47
				Potassium	%	1.97	1.21
Gross Energy	MJ/kg	15.2	15.5				
Dig.. Energy	MJ/kg	7.4	10.0	Iron	mg/kg	233	230
Met. Energy	MJ/kg	6.7	9.0	Copper	mg/kg	23	32
				Manganese	mg/kg	197	342
Myristoleic Acid	%	0.01	0.03	Zinc	mg/kg	153	437
Palmitoleic Acid	%	0.12	0.15	Cobalt	mcg/kg	10128	5207
Oleic Acid	%	1.50	0.82	Iodine	mcg/kg	15777	19236
Linoleic Acid	%	2.35	0.53	Selenium	mcg/kg	196	362
Linolenic Acid	%	0.47	0.36	Fluorine	mg/kg	40	30
Arachidonic Acid	%	0.10	0.08				
Clupenodonic Acid	%	–	—	Retinol	mcg/kg	80065	30951
				Cholecalciferol	mcg/kg	377	51
Lauric Acid	%	0.12	0.11	dl %Tocopherol	mg/kg	123	258
Myristic Acid	%	0.29	0.09				
Palmitic Acid	%	0.57	0.33	Vitamin B1	mg/kg	44.6	26.3
Stearic Acid	%	0.21	0.06	Vitamin B2	mg/kg	84.3	23.4
				Vitamin B6	mg/kg	21.7	17.3
Arginine	%	1.55	1.31	Vitamin B12	mcg/kg	1916.8	251.6
Lysine	%	0.41	0.84	Vitamin C	mg/kg	108.0	310.0
Methionine	%	0.15	0.29	Menadione	mg/kg	62.1	51.6
Cystine	%	0.14	0.25	Folic Acid	mg/kg	15.7	11.7
Tryptophan	%	0.14	0.25	Nicotinic Acid	mg/kg	237.4	62.1
Histdine	%	0.21	0.42	Pantothenic Acid	mg/kg	87.9	39.2
Threonine	%	0.37	0.65	Choline	mg/kg	1282.0	1330.0
Isoleucine	%	0.34	0.73	Inositol	mg/kg	722.0	1643.0
Leucine	%	0.60	1.18	Biotin	mcg/kg	217.0	483.0
Phenylalanine	%	0.40	0.83	p Aminobenzoic Acid	mg/kg	—	—
Valine	%	0.45	0.87				
Tyrosine	%	0.27	0.59	6 Carotene	mg/kg	99.2	49.5
Taurine	%	—	—	Carophyll Red	mg/kg	—	—

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