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# **Recent Research on Elephants and Rhinos**

Abstracts of the International Elephant and Rhino Research Symposium, Vienna, June 7-11, 2001

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# Preface

The present volume includes all Abstracts that were submitted to the International Elephant and Rhino Research Symposium in Vienna. This symposium was organized by the Tiergarten Schönbrunn in cooperation with the IEF (International Elephant Foundation) and the IRF (International Rhino Foundation), and was held from June 7-11, 2001. This marks the first time that the International Elephant Research Symposium was held outside the USA and the first time it was also combined with the Rhino Symposium.

The aim was to bring together scientists, researchers and elephant managers from the USA, Europe, Africa and Asia for an international scientific meeting in the heart of Europe, namely in Vienna.

A total of 57 oral presentations and 42 posters were accepted for presentation. The list of authors and topics shows that this objective has been met. It furthermore underlines that work is currently proceeding on a wide range of scientific topics. The spectrum extends from field conservation over nutrition, reproduction, health, behavior and the holistic Sri Lanka Project.

What has become abundantly clear is that both elephants and rhinos are highly endangered species that can only survive in specific protection areas. Their survival under human care is also less than certain, even though great strides have been made in improving their reproductive success. Nonetheless, the populations in the USA and Europe would rapidly age and decline without new imports.

The aim of the lectures and poster sessions at the Vienna symposium is to present and discuss the newest results as well as to point out remaining problems. This will be followed up by comprehensive workshops to develop working hypotheses and programs that will help improve the chances of survival for elephants and rhinos.

This abstract book is designed to serve as a reference volume and a working basis. Both the lectures and the posters are organized into chapters devoted either to elephant or rhino topics, whereby the abstracts themselves are arranged alphabetically according to author.

Harald M. Schwammer Vice Director Tiergarten Schönbrunn Vienna, June 2001

### Behavior and Serum Cortisol Concentrations of Three African Elephants Megan <u>Wilson<sup>1,2</sup></u>, Mollie Bloomsmith<sup>1,2</sup>, Maria Crane<sup>3</sup>, and Terry Maple<sup>1,2</sup> <sup>1</sup> TECHlab, Zoo Atlanta, Atlanta, Georgia, USA <sup>2</sup> School of Psychology, Georgia Institute of Technology, Atlanta, Georgia, USA <sup>3</sup> Zoo Atlanta, Atlanta, Georgia, USA

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Captive African elephants (Loxodonta africana) engage in a variety of complex behaviors, yet comprehensive research on these behaviors is limited. In addition, measures of cortisol have potential applications to elephant management practices that are currently understudied. The behavior of 0.3 African elephants managed in a free contact system was recorded using focal animal sampling. Proximity to other elephants and location in the exhibit were recorded, and all-occurrence data were collected on social behaviors. Data analyses indicated that the elephants spent much of the observed time feeding, primarily on provisioned hay. While in the outdoor habitat, the elephants were in areas visible to the zoo-visiting public a substantial amount of the observations. The elephants utilized all areas of the outdoor and indoor areas, with the exception of the pool. In addition, the elephants exhibited a variety of social behaviors, namely touching with the trunk the mouth or body of another elephant, and displacing of another elephant. Pushing, driving, and sparring also occurred. Weekly blood samples were collected from each elephant and serum cortisol concentrations were analyzed. The data provide important information about the behavioral repertoire and corresponding cortisol concentrations of captive African elephants. These baseline data will allow for meaningful comparisons when various management changes occur in the future.

### Is Musth a Reproductive Event? An Examination of Arguments for and Against this View

Presenter and PI: Lisa <u>Wingate</u>, DVM Co-Investigator: Bill Lasley, PhD University of California, Davis; (lwingate@ucdavis.edu)

Musth is a complex behavioral and physiological phenomenon occurring in sexually mature bull elephants of both genera. It is known that musth involves a dramatic elevation in androgens. Musth bulls exhibit a number of behavioral and physical changes. While some of these changes may serve as signals for conspecifics, or increase the bull's likelihood of finding estrous females, they occur at considerable cost to the bull.

There are a number of advantages which bulls in musth enjoy. Musth bulls automatically attain a rank higher than any non-musth bull. Furthermore, there is evidence that musth bulls have more access to estrous females, and are in fact preferred by them as potential mates.

However, it is not clear that musth is purely a reproductive phenomenon. The evidence is unclear on whether or not the reproductive axis (hypothalamic-pituitary-gonadal) is entirely responsible for the events of musth. Preliminary data from a pilot study will be presented which indicate increased adrenal activity during musth.

Alternative explanations for must will be explored, and an experimental design detailed to address some of the questions raised.

# **Oral Session – Rhinos**

### Long-Term Medical and Surgical Management of Chronic Pododermatitis in a Greater One-Horned Rhinoceros

Mark W. <u>Atkinson</u>\*, A. Rac Gandolf\*, Bruce Hull\*\*, Evan Blumer\* \*The Wilds, \*\* Ohio State University College of Veterinary Medicine (matkinson@thewilds.org).

This report discusses the ongoing treatment of an advanced case of bilateral, chronic pododermatitis in a 23-year old male Rhinoceros unicornis. Chronic pododermatitis is a poorly understood but common medical condition, which affects up to 28% of adult male R. unicornis in captivity. It generally affects the hind feet and is characterized by nonhealing fissures and ulcers located between the sole of the central toc and the adjacent pad and by pad overgrowth, bruising and chronic infection. Potential contributing factors include massive body size and weight, inappropriate housing conditions and husbandry techniques and possible genetic predisposition. The management plan for this animal includes changes in husbandry techniques and medical and surgical therapy. Medical intervention based on bacterial and fungal culture of lesions has included oral anti-microbial medication and topical application of copper sulfate/tetracycline by means of footbath. Surgical intervention involves regular debridement, aggressive trimming and paring of overgrown hoof tissue and the periodic application of collagen to stimulate granulation. To perform surgery on this animal, 24 chemical immobilizations have been performed during the past 55 months. Safe, repeated chemical restraint was achieved using combinations of etorphine, detomidine and ketamine injected intramuscularly. Intravenous ketamine supplementation following induction was utilized to improve immobilization and muscle relaxation and permit relatively invasive surgical procedures to be performed without significant or premature arousal. Changes in husbandry have included the addition of wood-mulch or cardboard substrate to the concrete flooring during winter holding, providing access to large areas of natural ground with free access to ponds and water and a diet that incorporates grass and natural browse. Regular assessment of hoof conformation and appearance is combined with thorough hoof trimming and nail care performed during anesthetic procedures. These changes in husbandry and the long-term therapeutic regimen have resulted in significant improvements in the appearance of lesions.

# Overview of IRF/ AZA Rhino TAG Conservation & Research

Evan S. <u>Blumer</u>, V.M.D. and Thomas J. Foose, Ph. D. International Rhino Foundation (IRF), 20 Pen Mar Street, Waynesboro, PA 17268, USA

There will be a brief overview of the status of rhino taxa (species & subspecies) in the wild and captivity. It will be emphasized that IRF & the AZA Rhino TAG believe both in situ protection and ex situ propagation are integral components of rhino conservation. The IRF & TAG have been cooperating for almost 10 years on contributions to both components and linkages between them. IRF supports and manages major in situ programs; the TAG coordinates the captive management and propagation programs in North America. The TAG has developed and periodically revised a Rhino Conservation Research Masterplan. Using this Masterplan, IRF has developed research priorities and supported research projects, largely selected through two competitive, peer-reviewed RFP (Request for Proposal) processes. In the last, RFP process, another organization SOS-Rhino also participated. Over \$1 million has been provided for rhino research. The emphasis in the research priorities and projects has been toward solving problems necessary to achieving viability of captive populations and programs as a component of rhino conservation strategies. The central problems, research priorities, and major projects will be briefly identified. Finally, it will be observed that many of the other presentations at the Symposium are reports from these research projects.

# Practical Applications of Operant Conditioning for Health, Research and Behavior in the Black Rhinoceros

### Christine M. <u>Bobko</u>, Denver Zoological Gardens (rhinoqueen@yahoo.com)

The use of operant conditioning for effective management of animals has been implemented with various species in the zoological setting. The principle behind this training technique is the use of positive reinforcement, which gives the animal a choice in participating in the behavior through a protected contact system. The Denver zoo training program was initiated due to an illness in a black rhino that required serial immobilizations for blood sampling. We initiated operant conditioning for the collection of biomaterials from our black rhinos as a comparatively non-stressful alternative to chemical immobilization. The training program targeted medical concerns which included blood and urine collection, rectal temperatures and footwork. Currently, a research project is underway to determine the correlation of environmental stressors and blood cortisol levels that will allow for improved management of this species in captivity. The application of operant conditioning has proved to be an invaluable tool for medical management, research and behavioral enrichment for our black rhinos.

# Sumatran Rhinoceros Conservation Strategy in Sabah

Edwin <u>Bosi</u> DVM, M.Phil<sup>1</sup>, Annelisa Kilbourn DVM<sup>2</sup> & Nancy Schaffer DVM<sup>3</sup> <sup>1</sup>Program Officer, SOS Rhino, c/o Institute of Tropical Biology and Conservation, University Malaysia Sabah, Locked Bag 2073, 88999 Kota Kinabalu, Sabah, Malaysia. <sup>2</sup>Field Scientist, SOS Rhino, c/o Sepilok Orangutan Rehabilitation Center, W.D.T. 200, 90009, Sandakan, Sabah, Malaysia. <sup>3</sup>Founder & President, SOS Rhino, 680 N. Lake Shore Drive, Suite 807, Chicago, IL 60611.

In 1993, the Asian Rhino Specialist Group (AsRSG) estimated between 48-68 Sumatran rhinoceros (*Dicerorhinus sumatransis harrissoni*) in the East Malaysian State of Sabah, north of the island of Borneo. The Wildlife Department of Sabah has indicated that there may be less than 30 individuals in the wild. The IUCN has placed this species as critically endangered.

In 1985, the Government of Sabah formed a technical task force in studying the presence, distribution of Sumatran rhino, and to undertake manage breeding. The rhinos relocated for captive breeding were from doomed areas or pockets of forest caused by agricultural development. Although Sabah was successful with breeding the rhinos in 1995, there was no success is producing any offspring. Captive management of Sumatran rhinoceros is challenging and only a pair is left at Sepilok of this subspecies. From 1995 to 1998, the Wildlife Department Sabah received substantial funding for rhino conservation from the Global Environmental Facility of the United Nation Development Program. In order to continue the conservation of Sumatran rhino in Sabah, SOS Rhino, a Chicago-based non-government, non-profit conservation organization has offered to assist the Wildlife Department of Sabah and began operating as of November 2000.

SOS Rhino has appointed a local consultant and program officer, a US-based wildlife veterinarian as field scientist and 12 local Rhino Field staffs. SOS Rhino is sponsoring one Malaysian student in University Malaysia Sabah to undertake a study on demography and nutrition of Sumatran rhino. Within a short period, SOS Rhino has successfully correlated the Progestrone level to estrus cycle to facilitate breeding. The first attempt has yielded encouraging result. SOS Rhino field exercise at the 120,000 hectare Tabin Wildlife Reserve has gathered tremendous amount of pictures of wildlife via phototrap cameras. The most vital findings were fresh tracks of Sumatran rhinoceros. SOS Rhino will be embarking on its community outreach program to involve the people living around rhino habitat to protect their heritage. SOS Rhino is also arranging special tour groups to have a first hand experience on wildlife in Sabah with a focus on the Sumatran rhino. The *in situ* and *ex situ* conservation works of SOS Rhino in Sabah is progressing very well.

We thank Mr. Mahedi Andau, Director of Wildlife Department Sabah for supporting our work in Sabah.

## Counting Rhinos From Dung: A Method to Estimate the Minimum Number of Animals Present in a Protected Area Using Microsatellite DNA

\*J <u>Cunningham</u>, A M. Morgan-Davies and \*C O'Ryan
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We report a DNA-based method to estimate the minimum number of individuals in a population of black rhinoceros, *Diceros bicornis*, in a reserve in southern Tanzania. The number of individuals present in this population could not be determined by conventional means for a number of reasons. In this pilot study, total genomic DNA was extracted from dung samples collected in the reserve and polymorphic microsatellite DNA loci were amplified using the Polymerase Chain Reaction. Although very low amounts of DNA were extracted, positive amplification products were obtained from 60% of the dung samples. For the remaining samples, plant inhibitors co-extracted with the rhinoceros DNA prevented the amplification of the microsatellite loci. Nine unique genotypes were observed using polymorphic black rhinoceros specific primers. Preliminary results suggest that, although the technique is not as yet reproducible, it provides the basis for non-invasive and cost effective sampling of rare and endangered animals in the wild.

### Development of a Pelleted Diet for Browsing Rhino Species Based on Native Plant Composition

Ellen S. <u>Dierenfeld</u>, James Jarzombek, Nancy A. Irlbeck, Robin Redcliffe, and John Fleming Wildlife Conservation Society, Nutrena Feeds, Inc., Colorado State University and Denver Zoological Garden, Fossil Rim Wildlife Center, and White Oak Conservation Center (edierenfeld@wcs.org)

The black rhinoceros (*Diceros bicornis*) is a browser in its native environment. Acquiring sufficient browse to meet the animal's feeding needs is difficult or impossible at most zoological institutions, and captive diets have typically been based upon concentrate and forage rations originally designed for domestic species. Such diets appear excessively digestible, and are often quite disparate in mineral, vitamin, fatty acid, protein, and carbohydrate constituents relative to native plants consumed. In an attempt to address some of the nutritional and physiologic needs of this browsing specialist, a wood-based (white oak, *Quercus alba*) pellet was formulated using the chemical composition of native browses as nutrient guidelines. Palatability studies were conducted at three facilities (n=5 black rhinos); intake and digestion information was collected at two locations (n=3 black rhinos). Blood samples were obtained to examine nutrient status pre- and post-feeding

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trials (n=5 black rhinos). All animals readily consumed the new product in addition to mixed legume/grass hay. Diet digestibility dropped from approximately 65% to 50%, a level similar to that measured in free-ranging rhinos eating native browses. Vitamin analyses indicated an overall rise in a-tocopherol levels (vitamin E) with the new pellet. There were no substantive changes in retinol (vitamin A) levels in any animals, and values for both vitamins were within normal ranges expected. Plasma fatty acid ratios, as indicators of recent fatty acid intake, appeared to be altered favorably by the addition of canola oil supplementation to the diet treatment and better duplicate those found in free-ranging rhinos. Modifications to the mineral and fatty acid content of the initial formulation were suggested from these preliminary data, and the pellet was further refined for commercial production. More extensive intake, digestibility, and investigations of physiological status are currently underway on a larger study group within the North American zoo black rhinoceros population.

### Red Cell Metabolism in the Black Rhinoceros: Relevance to Haemolytic Disease

### B.Weber, D.Paglia\* and E.H.<u>Harley</u>, Department of Chemical Pathology, University of Cape Town, South Africa, and \* Dept of Pathology and Laboratory Medicine, UCLA, USA

Captive black rhinoceros populations in the USA have been afflicted with a severe haemolytic anaemia syndrome, together with a leukoencephalopathy and other disorders suggestive of a free radical pathologic basis, and this has been the subject of intensive metabolic investigation for some years in our laboratories. The black rhinoceros (*Diceros bicornis*) shows a number of striking differences in its normal red cell biochemistry compared with humans: enzyme levels are often grossly different, ATP levels are 1/50th that of humans, and they contain very high levels of free tyrosine in their red cells (but not in plasma). On exposure to oxidative stress some tyrosine is converted transitorily to dityrosine, a substance never previously described in free form in cells, with an inverse relationship to glutathione levels. Human red blood cells incubated under the same conditions show no sign of dityrosine production.

Tyrosine is known to be a substrate for oxidative reactions, and has been implicated in contributing to defence against oxidative damage in seminal plasma. Experiments will be described which suggest that that tyrosine, together with some purine metabolites, are acting as an additional defence mechanism against reactive oxygen intermediates in red cells with marginal protective mechanisms. Oxygen radical absorbance (ORAC) assays, together with red cell tyrosine and purine levels, are currently being compared between in situ rhinoceroses in South Africa and captive (ex situ) individuals in Europe and the USA.. The integration of these in vitro and in vivo analyses should reveal insights and mechanisms exploitable for the development of preventative or therapeutic measures against haemolytic and other free radical induced disorders in these populations.

### Semen Collection, Sperm Assessment and Cryo-Preservation in African Rhinoceroses

R. <u>Hermes</u><sup>1</sup>, F. Göritz<sup>1</sup>, S. Blottner<sup>1</sup>, C. Walzer<sup>2</sup>, F. Schwarzenberger<sup>3</sup> and T.B. Hildebrandt<sup>1</sup> <sup>1</sup>Institute for Zoo Biology and Wildlife Research, D-10315 Berlin, Germany; <sup>2</sup>Salzburg Zoo Hellbrunn, A-5081 Anif, Austria; (chwalzer@eunet.at) <sup>3</sup>Institute of Biochemistry, University of Veterinary Medicine, Vienna, Austria.

The captive white rhinoceros population currently faces a demographic crisis. As a consequence substantial knowledge on reproductive biology of the female white rhinoceros has been gathered over the past years. However, little emphasis has been put on the evaluation of male fertility as a possible contributing factor to the low rate of reproduction. In the present study the reproductive fitness of ten male white and one black rhinoceros was evaluated by ultrasonography and semen assessment. Semen collection was obtained by manual stimulation (n=2) and electro-stimulation (n=9). Based on 39 semen assessment results seven males were identified as reliable semen donors. Preserved semen samples remained viable for up to four days. Cryopreserved samples showed post thaw motility suitable for assisted reproduction. Reproductive assessment provided accurate information on the breeding potential of male white rhinoceros with an implication on management decisions.

# AFLP as a Method for Genetic Wildlife Management in Rhinoceros

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Protection from habitat loss and poaching is not sufficient for rhino conservation, a specific breeding program should be applied as well. Since the migration of the remaining small populations is prevented, they can rapidly lose genetic variability and with it their capacity for genetic adaptation. To set up such a program, genetic information about the population structure is required.

The degree of inbreeding is primarily determined by the number of reproducing males in one area. Since very little is known about breeding patterns and mate choice in rhinoceros, genetic analyses can supplement behaviour observations.

Because the relationship of wild rhinoceros is not known, a molecular genetic approach was attempted to determine paternity and genetic variability. Lacking DNA sequence information, a suitable PCR method had to be found to generate genetic markers from the uncharacterised genome. We established the sequence independent DNA fingerprinting method termed "Amplified Fragment Length Polymorphism (AFLP)". To our knowledge, this is the first report of the use of AFLP markers to determine genetic relationships in wildlife.

A set of 64 AFLP primer combinations was analysed. Twelve primer combinations were selected for further investigation. They produced an average of 60-80 bands per animal per PCR reaction in a range of 50 to 510/800 basepairs. For all rhinoceros species, polymorphic bands could be detected.

The study involved 69 southern white rhinoceros (Ceratoterium simum simum) from zoological parks and a wild population of 57 animals. Additionally the study contained 5 northern white rhinoceros (Ceratoterium simum cottoni), 20 black rhinoceros (Diceros bicornis michaeli) and 6 great Indian one-horned rhinoceros (Rhinoceros unicornis).

For parentage testing a combined exclusion rate between 90 and 99% was reached. On a South African game farm with 5 white rhinoceros bulls, the most probable sire of 14 calves was determined. Simultaneously territorial status of males and behaviour of females was observed. The parentage test confirmed that females did not favour specific bulls. It showed that all males on the farm had reproduced. Courtship of a female did not guarantee fatherhood. The test reaffirmed that a female courted by a bull still mated with another male. This demonstrates that genetic analyses are important for accurate interpretations of the population structure and breeding success.

For the present, all rhino species still seem to show a high level of genetic variability, with an average heterozygosity of 0.36. This seems to be in contrast with reports for other species that have experienced near extinction.

### Digestibility Trials in the Zoo Applied to Field Studies of White Rhinoceros

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Nutrition has got a major influence on health, behavior and reproduction of animals, but only little is known about the nutrient requirement of white rhinoceros. In order to increase knowledge in this species a specific combination of zoo and field studies was conducted. The nutrient composition and the apparent digestibility of varied diets of a captive population was analyzed and compared with the feeding behavior of a free-living population.

The digestibility trials were carried out with a group of five white rhinos (1,4) in the zoo of Erfurt. Germany. The field study was conducted on free-living white rhinoceros in South Africa. Forage samples were collected from the feeding sites by following the tracks of three territorial males. Fecal samples of the same animal were collected two days later,

owing to the mean retention time of ingesta. Additionally, fecal samples of five males were collected over a period of one year. The samples were analyzed for dry matter, crude protein, crude fibre, nitrogen free extraction (Weender) and the cell wall constituents by Van Soest (ADF, ADL, NDF).

The mature grass in South Africa was higher in fibre and lower in protein content than the hay and grass feed in the zoo. The digestibility of nitrogen free extraction, crude fibre, organic matter and dry matter of the samples from South Africa was higher than expected, as in general a high fibre content is known to reduce digestibility. No differences were found in the quality of food and feces between territorial males during the rainy season. The fecal samples collected over the year showed a peak in the concentration of crude protein and cell constituents after seasonal rains.

# Population Growth, Sex Ratio and Reproduction of a Natural Living Population of White Rhinoceros

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White rhinos are being intensively managed both in captivity and in their natural habitat, but only a few data are available about the demography of this species. Informations about how the populations are regulated or patterns of their dynamics are however necessary in order to base management practices on ecosystem principles. In this study the demography of a natural living population of white rhinos was studied and the influence of management practices on the growth of the population has been analyzed. The study was carried out on a game farm in South Africa which housed a well growing population of white rhinoceros since 1991. All animals were individually known and their age was established by comparative horn and body analysis. The date of birth was determined by observations with  $a \pm 1$  month accuracy. Occasionally hunting of adult males and translocation of subadult males took place for management reasons.

The annual growth rate over the last 10 years was 15%. The high rate of increase is believed to be a consequence of the low population density (0.23 animals/qkm). The white rhinos reproduced seasonally with an increase in birth rates between December and June and a peak in March. The median interval between successive birth was 2 years and 3 months, however the length of the interval varied in dependence on the sex of the previous calf. Hunting of males has disarranged the adult sex ratio (15% males: 85% females), which is believed to have caused a skewed proportion of juveniles. More then twice as many males than females were borne during the last years. The high proportion of receptive females per male could possibly explain the shortened courtship period which was observed during the study. Whether the change in mating behavior has had an influence on the reproduction rate has still to be analyzed.

### An Overview of Diseases of Black Rhinoceroses in North America 1980 - 2000

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This report summarizes a number of diseases of unusual nature and uncertain etiology that have affected captive black rhinoceroses (*Diceros bicornis michaeli* and *D. b. minor*) in North America. Included are hemolytic anemia, fungal pneumonia, leukoencephalomalacia, several skin disorders including superficial necrolytic dermatopathy, idiopathic hemorrhagic vasculopathy, and hemosiderosis. The diseases have played a significant role in limiting the growth of that population. Hemolytic anemia is one example, in the past, it accounted for 40% of all adult deaths of captive black rhinoceroses (although its current incidence appears to be reduced). In contrast, a syndrome of mucocutaneous ulcers has had an even higher morbidity, but fortunately, a lower mortality.

Other conditions of note in captive black rhinoceroses also include an apparently high level of severe dental disease due to the presence of significant accumulations of dental tartar. In several black rhinoceroses ill from other causes, significant hypophosphatemia have developed. Liver failure from suspected creosote toxicosis has also been reported in both captive and recently imported black rhinoceroses. Several diseases, that are more commonly seen in domestic animals, such as tuberculosis, have also been reported.

In contrast to the black rhinoceros, the diseases of white rhinoceroses (*Ceratotherium simum simum*) in North America are of a more routine nature and apparently lower incidence. Efforts have been made to identify "common denominators" that may cause increased susceptibility of black rhinoceroses to some or all of these syndromes. A holistic approach is necessary as unusual patterns of cellular metabolism, hemosiderosis associated with time in captivity, various aspects of nutrition, and other factors are being evaluated to determine their relationship with these diseases. Additionally, a PhD candidate in veterinary epidemiology is surveying the health, nutrition and management of the North American population in attempt to identify further correlates with these syndromes.

# Microsatellite Analysis of African Black Rhinoceros (*Diceros bicornis*) to Determine Genetic Diversity and Population Structure

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The application of DNA markers coupled with the advent of the polymerase chain reaction has revolutionised the fields of evolutionary biology, population genetics and conservation biology. Molecular markers allow questions in biology to be addressed that could not be resolved by the more traditional means of morphology and behavioural studies. Microsatellite DNA consists of repeated units of short sequences and these hypervariable repeat loci are used extensively to quantify variation in populations. This study measures genetic variation and population structure in 107 black rhinoceros from three different populations or evolutionary lineages: 47 *D. b. minor*, 19 *D. b. michaeli* and 51 *D. b. bicornis*. Levels of heterozygosity, allelic diversity and genetic differentiation among populations were quantified using eight polymorphic microsatellite markers. There were high levels of genetic diversity in all three evolutionary lineages. Heterozygosity values ranged from 0.411 in *D. b. minor* to 0.718 in *D. b. michaeli*. Significant differentiation was detected among all pairwise comparisons done with an average Rst of 0.226. These results are discussed in the light of conservation management of fragmented black rhinoceros populations that are currently under threat from both increasing habitat destruction and poaching.

## Pathological Iron Overloads Acquired in Captivity by Browsing (but not by Naturally Grazing) Rhinoceroses

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African black rhinoceroses (Dicerosbicornis) in captivity are affected by a number of disorders of high morbidity and mortality, including acute episodic hemolytic anemia. Hemosiderosis, the deposition of iron pigments in multiple organs, has been the most consistent necropsy finding in this population and has most commonly been interpreted as evidence of previous hemolytic events. Direct participation in necropsies of black rhinoceroses dying in captivity, and review of histopathology of previous necropsies, revealed magnitudes and patterns of tissue iron deposition that were incompatible with hemolytic disease alone, but instead were indicative of a true iron overload syndrome that progressed in severity with time in captivity. This interpretation was supported by quantitative analyses of necropsy tissues and serum iron analytes, including sera from four of the five extant species of rhinoceroses and from both captive and free-ranging black and white (Ceratotherium simum) rhinoceroses. Significant, often extreme, elevations in serum and tissue iron and ferritin concentrations and transferrrin saturations were observed in captive adult black rhinoceroses compared to all control groups. Similar elevations were observed in the few Sumatran (Dicerorhinus sumatrnesis) rhinoceroses available for study, but not in the two species of natural grazers (African white and Asian greater one-horned [Rhinocerosnuicornis]). These findings suggest that iron homeostasis in browsing rhinoceroses may be dependent on natural iron chelators, such as tannins, phytate, mimosine, etc., that may not be included as components of formulated captive diets. Excessive iron stores may contribute directly and/or indirectly to several of the other serious disorders threatening this species in captivity, such as susceptibility to infections in general, to tuberculous and exotic fungal pneumonias specifically, and to acute and chronic anemia, toxic hepatopathies, and stress intolerance.

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Several semen procurement techniques, including penile massage, rectal massage, artificial vagina, and rectal probe electroejaculation were applied to four rhinoceros species. Penile massage, rectal massage, and artificial vaginas were applied to cognizant animals; rectal probe electroejaculation was applied to anesthetized animals. In some instances, multiple procedures were applied to individual animals. Success of these procedures was based on whether or not sperm was acquired. Penile massage was the direct manual massage of the penis. Rectal massage was manual manipulation of the accessory glands through the rectal wall. Artificial vaginas were applied to the erect penis during penile massage. Rectal probe electroejaculation utilized an ultrasound probe incorporated into a custom engineered electrical probe, designed by the authors. These procurement techniques were applied to a total of 21 animals. Penile massage was successful for all 5 black, all 5 white, 2 of 4 Greater One-horned Asian and 3 of 5 Sumatran rhinoceroses. However, only two animals, one black and one Indian, became trained to reliably produce samples with sperm. Rectal massage facilitated other methods, but was unsuccessful when used alone on 2 black, 1 white, 2 Greater One-horned Asian and 2 Sumatran rhinoceroses. An artificial vagina was applied to 2 black, 1 white, 2 Greater One-horned Asian and 1 Sumatran rhinoceroses, but was effective in only the black rhino that had been successfully trained for penile massage. Rectal probe electroejaculation was applied successfully to 2 black, 2 white and 1 Greater One-horned Asian. Seminal fluid was successfully collected in all 12 attempts to electroejaculate these 5 animals. Sperm was not present, however, in the fluids of one attempt in the Greater One-horned Asian. Rectal probe electroejaculation proved the most repeatable and reliable technique in producing seminal fluid with sperm.

# An IRF/SOS Rhino Sponsored Integrated Approach to Enhance the Reproductive Performance of White Rhinoceroses in the EEP

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Reproductive problems in white rhinoceroses include 1) acyclicity and variable estrous cycle lengths of 35 or 70 days, 2) mating failure due to acyclicity, or silent estrus due to sibling relationship/mate choice problems and 3) conception - pregnancy failure due to presumed uterine pathology. Reasons for these problems are still not definitively identified. The goal of this IRF-SOS Rhino funded multi-disciplinary, multi-institutional research is to work on possible solutions to overcome these reproductive problems. Our combined approach includes: 1) endocrine monitoring; 2) transfer of animals to enhance natural breeding; 3) clinical examinations of reproductive soundness in male and female white rhinos and 4) development of

artificial insemination (AI). Results of this ongoing study will be presented by several authors at this meeting. Findings reported were obtained from a large number of animals in different EEP institutions. Serial faecal reproductive monitoring includes >35 females and in the future will be extended to male white rhinos. Long term endocrine monitoring in female rhinos following a transfer between institutions indicates that estrous cycle activity could not be stimulated in some animals. This held true even 1 year after transfer and thus these animals will presumably not breed naturally. Monitoring of reproductive soundness to date includes 8 males and >15 females. Monitoring is done under full anesthesia or in deep standing sedation. This applied method has been successfully used >35 times and thus provides a reliable and safe immobilization protocol. Female reproductive tracts have been evaluated in >15 animals and beside reproductively sound animals, we have identified different types of flat liners, and animals with either uterine cysts or ovarian tumors. Male spermatological parameters after electro ejaculations have been evaluated in 8 males and have allowed to work on semen cryopreservation. In addition, work on conditioning 2 males for manual semen collection is currently underway. Different protocols for

ovulation induction were used and several AIs have been performed. Results demonstrate that a reliable ovulation protocol and an AI technique suitable to overcome difficult anatomical structures of the female genital tract is available. In conclusion, our combined approach including several institutions provides indispensable knowledge for the further propagation of the captive white rhinoceros population.

### Management of Reintroduced Greater One-Horned Rhinoceros (*Rhinoceros unicornis*) in Dudhwa National Park & Tiger Reserve, Uttar Pradesh, India

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The Greater one-horned Rhinoceros (Rhinoceros unicornis), which was once widely distributed from the foothills of the Hindukush Mountain Range (Pakistan) to Myannmar and also all along the flood plain of river Ganges. In last 200 years due to over hunting, fragmentation and habitats by cleaning forest for cultivation, disparate landuse for agriculture, extension of tea gardens, reclamation of grasslands and swamps for fulfilling the basic needs and other anthropogenic pressures were the major cause of elimination of Indian Rhinoceros from most of the former range of distribution. The last rhino in Uttar Pradesh (U.P.) was shot in the Pilibhit district adjacent to the Dudhwa National Park (NP) in 1878. Currently the Indian Rhino population of around 2,500 is restricted to protected areas (PAs) in Assam, West Bengal and Nepal. The Kaziranga NP in Assam has 1500 rhinos and the Royal Chitwan NP in Nepal 500 rhinos. The remaining rhino population with exception of Manas NP, India are surviving in small and insecure habitat patches with insecure future of survival. Despite of the protective measures and dedication of field managers and forest staff to protect the persecution of this animal continues due rising price of Indian rhino horn in the International market. By considering the current highly restricted distribution with poaching pressure, habitat specificity and in consideration to the scattered small population, to become imperative to reintroduce the species in suitable habitats in the former range of distributions. IUCN Rhino specialist group and The Rhino sub committee of Indian Board of Wildlife recommended the establishment of an additional rhino population and Dudhwa NP fulfill all the criteria required for the reintroduction and suitability. Translocation of nine Rhino from Pobitara WLS, Assam (5) and The Royal Chitwan NP, Nepal (4) to Dudhwa NP in the year 1984-85. Main aim is to highlight the performance of reintroduced rhino population in last 17 years, set backs and over all management, problems and future threats for a small population are discussed.

# Conservation Program for Sumatran and Javan Rhino in Indonesia, Malaysia and Vietnam

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The Javan rhino (Rhinoceros sondaicus) is the rarest, and the Sumatran rhino (Dicerorhinus sumatrensis) is the most vulnerable species of rhinoceros. Historically both species shared large parts of SE Asia from the foothills of the Himalaya in the north to the large Sunda islands in the south. Currently only scattered remnant populations remain in the wild, about 50-60 Javans' in two locations in Indonesia and Vietnam, and about 300 Sumatrans' in Indonesia and Malaysia. Although habitat reduction has played a role, the major reason for the decline is hunting for the horn and other body parts for traditional medicine. Rhino parts, in particular the horn, are a major ingredient in the Oriental pharmacopeia, and can fetch a very high price. Legal protection and creation of conservation areas has not been able to stop the poaching of rhinos and the trade in their parts, and both species could become extinct in the near future if not given more effective protection in their native habitats. Therefore the conservation programs for both species stress the establishment and operation of dedicated Rhino Protection Units (RPUs) in all major rhino areas. RPUs patrol rhino areas to prevent poaching, destroy traps, gather evidence and apprehend poachers and other trespassers in conservation areas. The RPUs are composed of regular wildlife officers and specially recruited and trained personnel and are funded by the respective governments with significant support from international donor agencies. Currently more than 200 persons are involved in RPU activities in SE Asia, protecting almost the same number of wild rhinos. Another component of the conservation program is managed breeding for future re-introductions. The Sumatran rhino breeding program started in 1985, but has not yet succeeded in reproducing the species under captive conditions although a pregnancy seems close.

### Anesthesia Management in White Rhinos for Reproductive Evaluation, Semen Collection and AI

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In order to clucidate the problems of poor reproductive performance in captive white rhinoceros (*Ceratotherium simum*).\* the EEP committee has encouraged intensive and serial reproductive monitoring in this species. Although the reasons for these problems have not been identified definitively, a multi-disciplinary, multi-institutional research proposal aims to work on possible solutions. During the period March 1999 to January 2001 a total of 37 anesthetic events were performed on 14 individual animals. All animals

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(estimated weight range 2000 – 2800 kg) were induced with a combination of Detomidine-HCL and Buthorphanol followed by Ethorphine - Acepromazine. In most procedures an additional i.v. application of Ketamine was applied as a "drop dose" in order to reduce the time to lateral recumbancy, and thus facilitate the correct placement of the animal within the enclosure.

A heavy-duty tire inner tube was placed beneath the shoulder in order to alleviate possible compressive trauma. All animals received supplemental oxygen at a rate of 15 l/min through a nasal tube. The mean duration of anesthesia was 76 ±48 min. Anesthesia was reversed in all cases with an i.v. combination of Naltrexone and Atipamezole Reversal was smooth and without signs of excitation. All animals were standing and alert approximately 2 min following administration of the antagonists. Once in lateral recumbency, rhino monitoring included measurement of heart by direct cardiac auscultation and respiratory rate by direct observation of thoracic excursions. The percent oxygen saturation of hemoglobin (SpO<sub>2</sub>) was continuously monitored using a hand-held pulse oximeter. Additionally sequential venous and arterial blood samples for monitoring purposes were drawn. The arterial blood samples were processed immediately with a portable blood gas analyzer. Prolonged recumbancy in white rhinos is associated with hypoventilation resulting in hypercapnia and respiratory acidosis. Through the provision of supplemental oxygen the severity of hypoxemia can be limited. Pulmonary shunting and ventilation/perfusion mismatch also likely play a role in recumbent anesthesia of the white rhino.

# Poster Sessions - Elephants

# Ultrasound Evaluation of the Pleura Space and Associated Connective Tissue in the Asian Elephant (*Elephas maximus*)

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The intimate connection between the lung and the chest wall of all extant elephant species is well documented. This relationship is critical in the anesthesia of elephants, necessitating that they be positioned in lateral recumbency. Clinical evaluation of the respiratory system is fairly limited at the present time and consistent of general health evaluations, blood gas determinations, trunk washes, and radiography in very young animals. More extensive diagnostics, such as bronchiole-alveolar lavage (BAL) may be feasible in given circumstances but would certainly necessitate a high-risk anesthesia. Thoracic ultrasound exams in clinically normal Asian elephants reveals a connective tissue layer of approximately 2cm with a fluid layer sandwiched between the parietal and visceral layers of 2-3mm. A culture positive elephant had findings that differed from the anatomy described in non-TB animals. Ultrasound evaluation may be a useful adjunct to pre-anesthetic screening once

# Poster Sessions - Rhinos

### Stimulation of Territorial and Mating Behavior by Fecal Samples. A Comparative Study on Behavior of Captive and Free-Living White Rhinoceros

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Urine and feces are being used for territorial marking in white rhinoceros. It is supposed that they carry information, which could indicate e.g. the presence of other individuals as well as their status and age.

White rhinos don't breed in captivity if they are kept in pairs. They are therefore being shifted between different enclosures. If it could be shown that excrements carry information, the presence of other animals could be simulating simply by olfactory markings. In the present study we analyzed territorial marking behavior of a free living population and explored whether olfactory stimuli could induce territorial and courtship behavior in captivity, by simulating the presence of other animals.

The zoo study was carried out with a group of five white rhinos (1,4) in the zoo of Erfurt, Germany. Fecal samples of males from different locations and age classes were introduced to the male and females and the changes in behavior and cycling activity were analyzed. The field study was conducted on a free living population in South Africa. Marking behavior was analyzed by following the track of individual males. The position of dung and urine markings was mapped with a GPS.

The results showed that males were more olfactorily interested than females. The introduction of feces from males (age 6-9) increased marking behavior of the male and social interactions between male and females. However 20-oxo-pregnane concentrations of the one cycling female did not change. In the free-living population males had no fixed border, their territories shifted in dependence on the presence of females. Urine was used to mark the territory border, whereas feces were found all over the area. This suggests that feces are more important for transferring information to females which could be confirmed by zoo experiments. Therefore we propose shifting feces between different enclosures rather than rhinos as an alternative management strategy.

### A Program of Manged Breeding for the Sumatran Rhinoceros at the Sumatran Rhino Sanctuary, Way Kambas National Park, Indonesia

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The Sumatran rhinoceros is the most critically endangered of the rhinoceros species with less than 300 individuals estimated to remain in the wild, a decline of more than 50% of the total alive 10 years ago. Furthermore the international effort to establish a managed breeding population for future re-introductions, has had limited success. Since its start in 1985 almost three-quarters of the animals have died and pairing and breeding has been problematic, with only one advanced pregnancy to date (Cincinnati, USA). As part of an effort to improve the chances for propagation by providing a more natural environment and social structure, the Way Kambas Sumatran Rhino Sanctuary (SRS) was founded in 1995. The sanctuary comprises 100 ha of mature secondary rain forest, located within the 125,000 ha Way Kambas National Park, Sumatra, Indonesia. The SRS provides a minimum of 20 Ha of native habitat for each individual and allows mixing and separation of animals as required. The founder animals, 2 females from Indonesian zoos and a male from the UK, arrived in 1998. Recently one of the females, an old and un-reproductive animal, died. The remaining pair is in prime condition and has breeding potential. Animals are monitored continuously during daylight hours, and full-time during the breeding periods, and extensive data on activity patterns, feeding and reproductive behaviour have been collected over the past 3 years. Morphological parameters and faecal and urinary hormone excretion patterns have been used to determine reproductive status of the females. For the latter purposes, a hormone assay laboratory has recently been established at the Centre for Life Sciences Study, Bogor Agricultural University. Initial attempts at non-invasive semen collection have also been carried out. This poster presents the results of 3 years of research and briefly discusses the contribution of the SRS to the conservation efforts for the Sumatran rhino.

## Comparison of The Antiphospholipid Syndrome to Medical Syndromes of Captive Black Rhinos (*Diceros bicornis*)

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Black rhinos in captivity have been plagued by a host of clinical entities. These include superficial necrolytic dermatitis (SND), hemosiderosis, hemolytic and non-hemolytic anemias, and most recently the idiopathic hemorrhagic vasculopathy syndrome (IHVS) has been described in a group of black rhinos. Other conditions affecting black rhinos include encephalomalacia<sup>10</sup> and necrotic laminar disease. Infectious conditions ranging from *Salmonella, Aspergillus* pneumonia, and leptospirosis have all been documented. Recurrent embryonic/fetal loss has been seen in one female by the author and in captive Sumatran rhinos (T. Roth, pers.comm.). The antiphospholipid syndrome (APS) is defined as the occurrence of venous and arterial thrombosis, recurrent fetal losses, and frequently a moderate thrombocytopenia in the presence of the phospholipid antibodies (aPL), namely lupus anticoagulant (aLA), anticardiolipin antibodies (aCL), or both. The pathogenesis of APS is quite simply thrombosis regardless of the organ system involved.

Comparisons between APS and black rhino syndromes may not be obvious at first but there may be some parallels (Table 1). Other manifestations and criteria that constitute APS in rhinos have been seen sporadically. Thrombocytopenia is a hallmark of the condition. Coagulation profiles and the development of black rhinoceros specific ELISA for APS related antibodies will be described.

Table 1. Comparisons within organ systems between APS and black rhino syndromes

System	APS	Black Rhino Syndrome
Skin/Digits	Cutaneous necrosis, livedo reticularis	Superficial necrolytic dermatitis, laminar necrosis, IVHS
Pulmonary	Pulmonary embolism	IVHS, hemosiderosis
Cardiovascular	Valvular lesions	Valvular hemosiderosis
Reproductive	Embryonic/fetal loss	Embryonic/fetal loss
Neurological	Cerebral ischemia	Encephalomalacia

# Multiple Isoflurane Anesthesia in a Captive Black Rhinoceros (Diceros bicornis).

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Inhalant anesthesia in rhinoceros has been reported on a couple of occasions. In all instances reported, euthanasia was performed or the rhino expired in the perianesthetic period from surgical complications. A four-year male black rhinoceros estimated at 1000kg was anesthetized twice and maintained on isoflurane and ventilated on two separate occasions for diagnostics evaluation of a fractured maxillary sinus. Combinations of detomidine, butorphanol, halperidol, xylazine, ketamine, and etorphine were used for pre-medications and inductions. A 28mm endotracheal tube was placed with the aid of a 1.7m colonoscope and isoflurane was administered with intermittent positive pressure ventilation. Serial blood gas taken from an auricular artery, non-invasive blood pressure monitoring with the cuff on the base of the tail, oxygen saturation via pulse oximetry, and base apex electrocardiogram (ECG) monitoring were followed during the procedure when feasible. The rhino was maintained in sternal recumbency for the entire procedure. The elapsed time of isoflurane anesthesia for the first anesthesia was 172 minutes and 230 minutes for the second procedure. Recoveries were smooth and uncomplicated with the animal exhibiting normal behaviors within 2 hours of each event.

### Vocal Communication of the Black Rhino Diceros bicornis ssp.

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Although rhinos have been the focus of many studies not much is known about their communication. A more detailed study has been done on the vocalizations of the Indian Rhino but not on the other rhino species. It was also suspected that all rhino species use infrasound signals for their communication but there is no detailed scientific investigation with a sufficient number of rhinos. This study investigates the audible vocal communication of the Black rhino Diceros bicornis ssp. and if there is evidence for the use of infrasound signals. The project also tests if individual identification by acoustic means is possible. Following zoos were visited for the investigations: Zoo Frankfurt with 1,1 Diceros bicornis michaeli. Recordings were performed with a Sony DAT recorder TCD D100 and a Sennheiser directional microphone. Acoustic data were evaluated with the help of the

Software Avisoft. Following parameters were measured: duration, minimum frequency, frequency with the highest energy, maximum frequency. Behavioural data were collected at the same time when recording. Following calls could be identified: Begging call, snort, hollow snort, growl, begging call of juvenile one year old and begging call of juvenile ten days old. There was no evidence for vocalizations in the infrasound range only. It can however, not be excluded that wild rhinos use infrasound for finding mates. Recording of wild rhinos was not possible in the frame of this study.

### Feeding Tannins to Captive Black Rhinoceros (*Diceros bicornis*): Results of a Pilot Study

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It has been postulated that the lack of tannins in captive diets for black rhinoceros (Diceros bicornis) might be responsible for the occurence of iron overload in captive specimens. In theory, the presence of iron-chelating tannins in natural diets might have induced the evolution of an especially effective iron absorption mechanism in the species, which would lead to excessive iron uptake in the absence of such chelators. In order to investigate this problem, 5 captive rhinos were fed two diets, one with and one without a tannic acid supplement. Feed intake and faecal output were measured for two 5-day periods, respectively. Additionally, the use of several markers (Co-EDTA, Cr-mordanted fibre, nalkane C36) as pusle-dose and steady state markers was assessed. The animals accepted the tannic acid-supplemented food without hesistation. Measured passage rates suggest that a 4-day collection period should result in total recovery of a pulse-dose marker. Iron concentration of faeces did not differ significantly between treatments and was apparently influenced by soil intake from the enclosure. We conclude that it is feasible to produce tannin-containing feeds for the further evaluation of iron metabolism in black rhinos. Either animals should be kept indoors during trials to prevent soil intake, or an iron isotope should be applied as a pulse-dose marker to compare recoveries between treatments. Additionally, we will present data on digestibilities and marker recoveries.

### **Rhino Translocation in Nepal**

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Since 1986, conservation organizations in Nepal are involved in rhino translocation to safeguard greater one horned rhinoceros (Rhinoceros unicornis) population. The rhinoceros from the Royal Chitwan National Park (RCNP) were transported and released in Royal Bardia National Park (RBNP) and Royal Suklaphanta Wildlife Reserve (RSWR) in various occasions. The aim of these translocations is to establish new populations of this megaherbivore within Nepal. Similarly, objectives of the translocation programs are to reduce the risk of population extinction from any disaster in their single home & to minimize human-rhino conflict in the mother gene pool. Altogether, 62 (26 males and 36 females) rhinoceros were translocated in various years (1996, 1991, 1998, 1999 and 2000). Out of total translocated animals 58 (24 males 34 females) were released in RBNP and 4 (1 male and 3 females) in RSWR. The overall translocation activity can be categorized into three main steps. Firstly, pre translocation arrangements include the preparation of cages, selection of site for capturing, identification and determination of age and sex of the translocating animals and habitat assessment in the released site. Secondly, the translocation process starts by capturing and loading into the cage and truck and transported. Thirdly, the post translocation activity includes the monitoring of translocated animals to observe their behavior in the new habitat. The whole operation of translocation is planned and executed by the group of Nepalese experts, conservation professionals and wildlife technicians. The monitoring report of these rhinoceros indicates that these animals are well adapted in their new habitat.

### Feeding Ecology of Desert Dwelling Black Rhinoceros Diceros bicornis L. in Kunene Region, Namibia

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Feeding ecology of black rhinoceros was studied in extreme arid Kunene region Nordwest of Namibia (mean rainfall <150 mm/year), during the dry season from June to October 2000. Using the feeding track method showed, that rhinos were browsed on (about)\* 65 of (about)\* 140 encountered species (Loutit et al. (1987) 70 feeding species out of the total of 101 species). After the very good rain season 1999/2000 rhinos were feeding beside shrubs and woody species also on a high variety of herbs. The most heavily used feeding specie was the herb *Chamaesycae glanduligera* (Euphorbiaceae).

In comparison to the examination of Loutit et al. the study area varied slightly in a higher population density of black rhino and lower desert influence.

\* Please note: At submission date identification of plants and data evaluation was still in progress

# Reproductive Disorders in White Rhinoceroses and the Value of Ultrasonographic Assessment

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Based on endocrine monitoring 50% of the female white rhinoceros EEP population is considered to have no luteal activity. This lack of luteal activity represents one significant cause for the limited reproductive success of white rhinoceroses in captivity. Ultrasound evaluations of the reproductive tract combined with long-term endocrine monitoring were conducted in n=11 an-oestrous and 4 cycling females to elucidate the ovarian and uterine status. Five sonographic types of an-estrous (flat-lining) females were characterised: type *I* (n=3) small inactive ovaries, associated with uterine fluid accumulation in one older female; type II (n=2) persistent luteal structures; type III (n=1) animal with follicular cysts, type IV (n=4) young females with dynamic follicular development but lack of ovulation, type V (n=1) animal with massive para-ovarian tumour. In three regular cycling females cystic endometrial alteration was identified as predominant pathological finding. Due to the advanced status of alterations, a total of four females ( $\geq$ 27 years) were considered post-reproductive. Only females <11 years were free of pathological alterations. Reproductive tract ultrasound resulted in (1) the accurate evaluation of the breeding potential, (2) the sonographic characterisation of an-oestrous females and (3) the selection of candidates for natural or artificial breeding efforts. Increased use of ultrasonography in rhinoceros reproduction is useful tool to enhance breeding success in captivity.

### Using Daily Keeper Questionnaires to Predict Estrus in Rhinos

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Recognizing and understanding the basic biological characteristics of reproduction in rhinos will help zoo managers facilitate their propagation. The Daily Keeper Questionnaire (DKQ) is part of a larger longitudinal research project on reproduction in African rhino species and is being tested on Zoo Atlanta's black rhinos (*Diceros bicornis*) to identify which behavioral and physical characteristics are the most reliable indicators of estrus. Various versions of the DKQ were developed as management tools to assist animal care staff in identifying and following estrus, gestation, and parturition in African rhinos. Predictive ability can allow staff to advantageously prepare for these reproductive events. The current version of the questionnaire for estrus has 15 behavioral and physical attributes of adult male and female rhinos related to reproduction. The items on the questionnaire are rated on a 0-4 scale and pertain to both the female and her mate(s), as the latter may be providing

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indicators of the female's state. The DKQ was designed to capture the keepers' general impressions of the rhinos without requiring time away from daily routines for formal observations and takes only five minutes to complete at the end of each day. Preliminary results indicate that estrus prediction is possible for individual rhinos using this DKQ. Analysis of Zoo Atlanta's female's data suggests that increased agitation, interest in the male, aggression, and vulvar swelling or winking contribute most to keeper recognition of impending estrus. For the male, the most salient contributing factors are increased aggression, vocalization, agitation, horn digging, and following. Chin resting and mounting are strong factors which are indicative, though not predictive, of estrus. By participating in behavioral data collection, the keepers are generally more aware of the rhinos' changing dispositions, allowing improved daily management of the rhinos including preparations for breeding.

# Faecal steroid analysis in free-ranging female white rhinoceroses (*Ceratotherium simum*) on Otjiwa game ranch in Namibia

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Acyclicity is the major cause of low reproductive rates in captive white rhinoceroses; additional confounding factors are variable oestrous cycle lengths of 35 or 70 days and silent oestrus due to mate choice problems. The objectives of this study were to determine whether these conditions are present in wild populations. Study site was Otjiwa game ranch, Namibia (100 km<sup>-</sup>; n=26 white rhinoceroses). Fresh dung samples were serially collected after tracking by game scouts. Samples were stored in methanol and dried prior to analysis with an established 20-oxo-pregnane assay. All females of breeding age (n=8) had calves; calving intervals of less than 2.5 years in the older animals (n=6) indicate that infertility conditions are not present in this wild population. Farm size and brush coverage made tracking difficult and average individual sample collection interval was  $38.5 \pm 5.0$  d (range 1-100 d). This and the fact that females seem to become pregnant shortly after lactational anoestrus hindered statements on cyclicity. However, pregnancy diagnosis, even from single samples was 100% accurate after Day 120 (threshold value: 3000 ng/g faeces; significantly higher concentrations in >120d pregnant versus non-pregnant and <120d pregnant animals; p=0.001). In summary, pregnancy was accurately diagnosed by faecal steroid analysis in a wild white rhino population. Low reproductive rates, similar to those in the captive population were not present. For oestrous cycle diagnosis sample frequency during inter pregnancy periods needs to be increased. It can be concluded that faecal steroid analysis in wild white rhinoceros populations offers new perspectives for in situ and exsitu management of this species.

## Progesterone Supplemention and Pregnancy in a Black Rhinoceros (Diceros bicornis): A Case Report

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A wild-born (1962 est.) female black rhinoceros (SB#53) at the Sedgwick County Zoo maintained four pregnancies with medical management and progesterone supplementation. She had produced two live offspring 15 years earlier, but, after breeding to the same male (SB#301), she aborted three times (8 months (Oct. 1989), 10 months (April 1991), and 4 months (December 1991)). Her reproductive status was monitored through excretory hormones and ultrasonography (Berkeley et al. 1997). Oral progesterone (2.2 mg/ml Regumate, Hoechst-Russell) was administered 15 ml/ BID from the 2nd or 3rd month of pregnancy to a gradually reduced dose during the assumed last month of pregnancy. The caloric and vitamin/mineral content of her diet was supplemented and hay shredded for digestibility. Medical problems were addressed as indicated by weekly analysis of hematology and serum chemistries. Mucopurulent vaginal discharges, periodic skin ulcerations, imbalance in the calcium to phosphorous ratio, low glucose and sand impaction were treated accordingly. She consecutively, delivered a male calf (1993), aborted in the last month (1996), delivered a female calf (1999) and then died pregnant (2000). Maintenance of four pregnancies over eight years was aided with intensive medical and reproductive management.

Berkeley, E.V.; Kirkpatrick, J.F.; Schaffer, N.E.; Bryant, W.M.; Threlfall, W.R. Serum and fecal steroid analysis of ovulation, pregnancy, and parturition in the black rhinoceros (Diceros bicornis). ZOO BIOLOGY 16:121-132, 1997

### Utero – Ovarian Pathological Complex of the Sumatran Rhinoceros (Dicerorhinus sumatrensis)

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Sumatran rhinoceros reproductive biology is complicated, and has contributed to the failure of this species to flourish in captive breeding facilities. Physiological failures have included absence of intromission, intromission lacking conception and reabsorption. Twenty-two females have entered captivity since 1984. Seventeen of these animals were examined and at least 50% exhibited reproductive tract pathology. Uterine tumors and cysts were noted with ultrasonography. Leiomyoma and cystic endometrial hyperplasia was reported postmortem. One female exhibited 8-year-long galactorrhea and another an unusual

occurrence of multiple corpora lutea on the ovaries and enlarged uterus. Pathology appears to begin after 10 years of age, assuming animals were mature when captured, and becomes prevalent in animals over 15 years old. Of the remaining ten females in captivity, eight are copulating. Three have no pathology, and the other five are copulating with unknown to mild or significant pathology. These animals will have increased difficulty conceiving, though they continue cycling. The etiology of these pathologies needs further investigation.

### Manual Semen Collection in a Standing Rhinoceros (Ceratotherium simum simum)

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Reproductive technologies, such as artificial insemination, require routine methods of semen collection. As the common technique to obtain semen samples from rhinoceros is electroejaculation, the objective of this study was to develop a less invasive procedure without the necessity of anaesthesia in order to perform repeated deseminations. Based on case reports in the equine literature on successfull pharmacologically induced ejaculation in stallions using alpha-2-agonists, a study with two white rhino bulls (age 20 and 30) at Salzburg Zoo Hellbrunn was undertaken using Detomidine (Domosedan?) and a combination of Detomidine (DomosedanO) and Butorphanol (Torbugesic?) applied IM. To evaluate the minimum dosage necessary, various dosage-related protocols are being performed. In- and outdoor restraint chutes enable easy access to the genital region of the white rhino bulls while providing the highest standard of safety for the operating person. First experiences demonstrate that a dosage of 14 mg Detomidine / bull / session regularly induces a reversable penile protrusion approximately eight minutes after the injection followed by a medium erection of the penis without manual stimulation. Massage of the preputium then leads to a full erection with cranioventral orientation of the penis and pulsatile contraction of the penile muscles. Ejaculates could be obtained using a dosage of 14 mg Detomidine, but were of poor volume and sperm concentration and were highly contaminated by urine. Whether a complete ejaculate with acceptable sperm quality and quantity can be collected by the combination of Detomidine and Butorphanol still has to be investigated in this study. In comparison to manual stimulation without pharmacological induction we can conclude that IM applied alpha-2-agonists induce stronger erections of longer duration. Additionally due to the slightly sedative effect general manipulation of the restrained rhinoceros is facilitated.

### Genetic Kinship and Social Structure in White Rhinos at the Zoological Center Tel Aviv -Ramat Gan Israel

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Since 1974 the Zoological Center Tel Aviv- Ramat Gan has kept a large herd of 11-15 white rhinos in a free ranging multi-male situation. The total number of adult males ranged between 4-6. Over the years 14 rhinos were born, the most recent in 1996. A drop in fertility, including first generation animals led us to investigate whether genetic and behavioral components contribute to the problem. Since multiple male matings occur, paternity can only be determined genetically. During 1998-1999 hair samples were taken from all 11 living rhinos at the Safari. The method of DNA analysis was random amplified polymorphic DNA (RAPD).

Paternity was identified in 5/7 offspring present. Three young were fathered by one male, and one each was fathered by two other males. The identities of fathers of two other calves is unknown, and could have been males that have died or are no longer in the Safari. Behavioral observations on social preferences and copulatory behavior were made over the 2.5 year time period. Stable social preferences were found among the females, not necessarily related to matriline. The same male who fathered the most young is solitary while the other males form loose groups. Further observations showed that both males and females could interfere during copulations.

The results have led us to continue behavioral studies, and following fertility evaluation, planned for May 2001, we will reconsider our management system.

### Demographics of Sumatran Rhinoceros (*Dicerorhinus sumatrensis* harrissoni) in Sabah, Malaysia : Correlation to food distribution in Tabin Wildlife Reserve

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The Sumatran rhinoceros (*Dicerorhinus sumatrensis*) is the most primitive and smallest species in *Rhinocerotidae* family (Groves, 1967). This animal is included in one of the 12 most endangered species in the world according to the 'International Union for Conservation of Nature and Natural Resources' (IUCN) data. Sumatran rhinoceros in Sabah are fully protected under the Wildlife Conservation Enactment 1997. The Borneon subspecies of Sumatran rhino can be found in several locations principally in the east of Sabah, Malaysia (Payne, J et al. 1985). Their distribution was focused account for

residencies which are the Residency of Sandakan and Residency of Tawau especially in the Kinabatangan district in Sandakan and Lahad Datu district in Tawau. Recent reports from the 'Asian Rhinoceros Specialist Group of SSC (Special Survival Committee)' from IUCN in 1989 believe that the population in Sabah may be as low as 30 individuals (Yasuma & Andau, 1999). This species is becoming extinct because of poaching activities for their valuable horn, believed to have medicinal value. Their future survival is also in jeopardy due to habitat loss and disturbance. This species may only be found currently in protected areas such as Tabin Wildlife Reserve. The 1998 Tabin scientific expedition reported that at least three individuals were found in their study area consisting of 8000 ha. of primary rainforest. Jomitin (1999) reported that one of their survey groups were face to face with Sumatran rhino, which very rare occurrence. Unfortunately, the animal fled prior to being photographed. According to the size, age of and foot print location, 3 individuals could be confirmed in their study area. Due to the elusive nature of this animal, the dense vegetation and relief of their habitat, most estimates of population size are based on the presence of footprint or dung samples and very rarely on actual animal sightings. Information on the demographics of these animals in the wild and home ranges of individuals is therefore very limited. Using new tools such as camera traps, GPS systems, vegetation evaluation, genetic analysis and GIS mapping, we hope to acquire additional information to help determine the composition of the remaining wild population of rhino in Sabah. Hence, in the future we hope to predict the carrying capacity of the various habitats for Sumatran rhino based on food supply. The results from this survey will produce objective data used to assess the status of these animals and make well-planed future wildlife management decisions to assure their survival into the next millennium.

An Overview of Pathological Alterations of Hooves and Soles of Captive Indian Rhinos (*Rhinoceros unicornis*) and a Comparison of Anatomical Foot Structures of Captive and Wild Indian Rhinoceroses

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Captive Indian rhinos (*Rhinoceros unicornis*) suffer from numerous pathological alterations of their feet structures. Cracks between the pad and the central sole are a common finding. All breeding bulls (n=11) and more than half of the breeding females (n=8) of the investigated European population (13.19) suffered from these cracks with various degrees of severity. 69% (n=22) of all rhinos had abraded side horn walls and 41% (n=13) showed vertical and/or horizontal cracks in the horn wall with inflammatory processes along the coronary band. 16% (n=5) showed fistules and ulcers in the pad. All feet structures were examined histologically, using the feet (n=6) of deceased animals and horn samples collected from pads, soles, and horn walls during foot care procedures. The histological results as well as the macroscopical findings were compared with the feet of wild animals (n=10). The comparison of captive and wild feet revealed that wild Indian rhinos have long hooves, with the sole protruding the pad for several centimetres. The rim of the central sole adjacent

to the pad is elevated and all soles have a concave shape. The horn of the pad appears rough, hard, and free of pathological alterations. The feet of captive Indian rhinos have a very smooth pad surface with the horn layer being 1cm thick at the most. The soles of the three hooves have a flat and even surface; the hooves are short. The pad and the central sole adjoin at even level. The comparison of wild and captive animals show that wild rhinos are by nature 'sole-walkers', captive animals show abraded horn structures and turned to 'pad-walkers'. These abraded hoof structures are prone to acute or chronic impacts. It is suggested to reconsider some husbandry aspects to prevent the occurrence of foot problems in future generations of Indian rhinos.