

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

R. Hermes¹, F. Göritz¹, S. Blotner¹, C. Walzer², F. Schwarzenberger³
and T.B. Hildebrandt¹

¹Institute for Zoo Biology and Wildlife Research, D-10315 Berlin, Germany;

²Salzburg Zoo Hellbrunn, A-5081 Anif, Austria; (chwalzer@eunet.at)

³Institute 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

Katharina Kellner¹, M. Förster¹, P. Kretzschmar²

¹Institut für Tierzucht der Ludwig-Maximilians Universität Munich, Veterinärstr. 13,
80539 München, Germany, katharina_kellner@web.de

²Zoological Institute I, Friedrich-Alexander University of Erlangen-Nürnberg,
Staudtstr. 5, 91058 Erlangen, Germany

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 (*Ceratotherium simum simum*) from zoological parks and a wild population of 57 animals. Additionally the study contained 5 northern white rhinoceros (*Ceratotherium 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

B. Kiefer¹; Kretzschmar, P.²; Ganslosser, U.²; Lechner-Doll, M.³; Kienzle, E.¹

¹Institute of Physiology, Physiological Chemistry and Animal Nutrition, Faculty of Veterinary Science, Ludwig-Maximilians-University, Veterinär Str. 13, 80539 Munich, Germany, (breitmaulnashorn@gmx.de);

²Zoological Institute I, Friedrich-Alexander University of Erlangen-Nürnberg,
Staudtstr. 5, 91058 Erlangen, Germany;

³Institute for Zoo Biology and Wildlife Research, Berlin, PF 601103, D-10252 Berlin, Germany

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

Kretzschmar, P.¹; Ganslosser, U.¹

¹ Zoological Institute I, Friedrich-Alexander University of Erlangen-Nürnberg, Staudtstr. 5, 91058 Erlangen, Germany (Petra.Kretzschmar@gmx.de)

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 ± 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 than 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

R. Eric Miller, DVM, Dipl. ACZM,
Saint Louis Zoo (E.MAIL: remiller@stlzoo.org)

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.

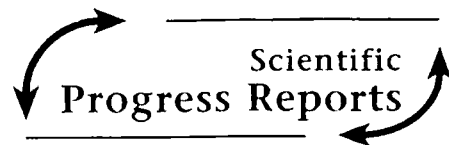
In contrast to the black rhinoceros, the diseases of white rhinoceroses (*Ceratotherium simum simum*) in North America are of a more routine nature and apparently lower incidence. Efforts have been made to identify „common denominators“ that may cause increased susceptibility of black rhinoceroses to 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

Colleen O’Ryan*, Jessica Cunningham*, Ingrid Baumgartner**
and Eric Harley**

*Molecular and Cell Biology, University of Cape Town, Private Bag, Rondebosch, 7700, South Africa, **Chemical Pathology, Medical School, University of Cape Town, Anzio Road, Observatory, 7925, South Africa
corresponding author: colleen@molbiol.uct.ac.za

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



Harald M. Schwammer
Thomas J. Foose
Michael Fouraker
Deborah Olson



Recent Research on Elephants and Rhinos

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