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USE OF DNA TECHNOLOGY IN WHITE RHINO (*CERATHOTHERIUM SIMUM*) IDENTIFICATION AND ITS APPLICATIONS IN CONSERVATION

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ABSTRACT

South Africa plays a critical international role in white rhino, Cerathotherium simum *conservation. The Mpumalanga Parks Board (MPB) is the statutory organization entrusted with conservation in the Province. The MPB hosts key populations of white rhino on some of its protected areas. None of these populations is currently or potentially large enough to be viable on its own. Challenges for an expansion in numbers are the limited habitat available and intra-specific competition. A meta-population strategy is required to overcome these challenges and to prevent inbreeding. The effective management of small sub-populations within a meta-population strategy requires the identification and permanent recognition of individual rhino. This has been achieved in the recent past through micro-chipping, ear-notching and the compilation of an individual file for each animal. Despite the intensive documentation and monitoring of these sub-populations, the extent to which individual bulls are responsible for successive generations of calves cannot be established with certainty. Genetic data are required. A DNA profiling technique based on molecular genetics was developed by the Animal Improvement Institute of the Agricultural Research Council using material collected from the MPB rhino. Individual animals can now be unequivocally identified by means of unique bands of micro-satellites. Each individual profile consists of a combination of the two parent's profiles, making it therefore possible to construct parental lineages. A genetic database of the MPB rhino is being constructed. The information from the database is being applied to determine parentage, animal productivity, genetic variation, rate of inbreeding and genetic distances between populations. The DNA profiling technique provides a powerful tool for the MPB to preventing deleterious genetic consequences of maintaining small, isolated sub-populations. Objective decisions can now be made as to which individual rhino should be exchanged amongst the different sub-populations.*

I. INTRODUCTION

White rhino, *Cerathotherium simum*, together with the black rhino, *Diceros bicornis*, are two of the most charismatic mega-herbivores left on our planet and have become flagship species for international conservation. They are significant not only for the continuation of a major evolutionary heritage but also as symbols for the protection of African savannas (STUART, 1999).

The survival of the white rhino is not yet assured. The latest IUCN Red Data Mammal Book lists the species as "Near Threatened" (FRIEDMAN and DALY, 2004). Although the numbers have been steadily increasing in South Africa, the species is still conservation dependent and at risk to qualify as threatened with extinction again, should conservation measures be withdrawn. South Africa plays a critical international role in the conservation of the southern white rhino subspecies. The country currently holds 34 of the 77 southern white rhino populations (77%) rated as being 'key' or 'important' (EMSLIE and BROOKS, 1999).

The mission of the African Rhino Specialist Group (AfRSG) is to promote the long term survival of Africa's rhino populations and to provide funding for research projects. Populations with the highest conservation value are categorised as "key" (critical for the survival of the subspecies) or "important" (extremely valuable for the survival of the subspecies). The conservation value of a population is determined by relevant parameters, including population size, significance of the population in conserving the subspecies, and the likelihood of the protection/conservation measures being effective. Donors use this conservation rating of populations as a guideline in determining priorities for project funding.

The Mpumalanga Parks Board (MPB) is the statutory body responsible for biodiversity conservation in the Province of Mpumalanga. It is also to ensure the proper conservation and management of the Protected Areas within its portfolio (STALMANS and STEYN, 2001). The southern white rhino, *Cerathotherium simum simum*, occurs on a number of the Protected Areas (Figure 1). According to this AfRSG classification, the MPB has two Important 1 Populations. That is a "population that is increasing/stable and $n = 20$-50". Other populations in the MPB are even smaller. However if all MPB rhino are considered being part of a single meta-population, they could together be classified as a Key 1 Population. This is a "population that is increasing/stable, $n > 100$ rhinos, or 50% of the subspecies". A Key 1 population has importance for conservation and when applying for international funding. The Rhino Management Group of southern Africa advises that it is wise to have species with conservation priority in more populations under a range of management models. The MPB therefore needs to increase populations in order to address its conservation responsibility. This will simultaneously improve the economic value and tourism potential of reserves.

II. META-POPULATION MANAGEMENT STRATEGY

The MPB faces significant challenges to increase the size of individual rhino populations in each Protected Area. These challenges include the limited

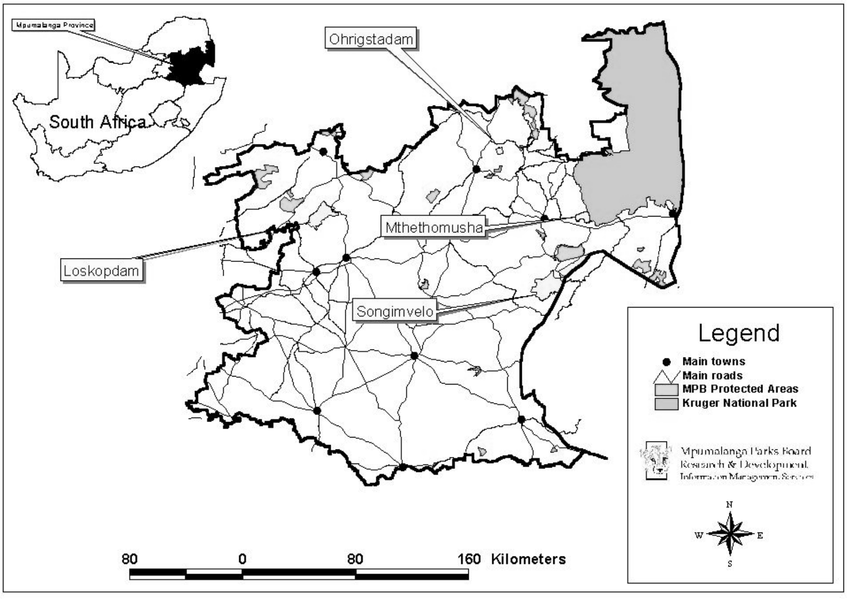

Figure 1: Location of the Mpumalanga Province in South Africa (top left) and of the Protected Areas of the Mpumalanga Parks Board that have white rhino, *Cerathotherium simum*, populations (in grey, except the Kruger National Park in the north-eastern corner of the Province).
Figure 1 : Localisation de la Province de Mpumalanga en Afrique du Sud et des Aires Protégées du Bureau des Parcs de Mpumalanga qui ont des populations de rhinocéros blancs, *Cerathotherium simum* (en gris, à l'exception du Parc National Kruger dans le coin nord-est de la Province).

suitable habitat that is available and intra-specific competition. More than 50% of mortalities on the protected areas are as a result of territorial fights between bulls. The other causes of mortalities are drowning, falling of cliffs, capture, and poaching.

The long term survival of a species depends on the maintenance of as much genetic diversity as possible (EMSLIE and BROOKS, 1999). Genetic concerns in small populations relate to inbreeding, loss of genetic heterozygosity and associated loss of potential adaptability to the environment, and disease resistance. This loss is non-adaptive. Genetic diversity is important for the maintenance of fitness of a population. Variance in environmental conditions impacts upon reproduction and survival. Small populations can be viable if managed as a single larger meta-population. This effectively increases the size of the total number of the population and improves the chances of survival following a stochastic event (SWART and FERGUSON, 1994). Individual animals can be moved between sub-populations of the meta-population to correct genetic and demographic problems. The movement of animals would require the migration of at least one breeding animal per generation (approximately every 12-17 years) (EMSLIE and BROOKS, 1999).

A meta-population approach, whereby individual sub-populations form part of a single, larger population can thus overcome the genetic concerns. Within sub-populations, the individual monitoring of rhino, the assessment of the contribution that individual bulls make to the siring of calves, and the determination of the productivity of individual cows can go a long way to increase the effective size of a population without necessarily increasing absolute numbers. In this way, available habitat can be optimally stocked.

Key to the meta-population approach is the recognition of individual animals. The identification of sub-adults in particular is difficult and can lead to over/under estimation of population size. Permanent recognition of each single individual rhino and documenting its performance has so far been achieved through earmarking and micro-chipping when a sub-adult. This ensures positive identification in the field, as well as linking poached horns with specific individuals/localities. There is also an increased chance of convictions in court and an increased value of monitoring data.

Despite this intensive monitoring effort, it remains difficult to determine with certainty which bulls are responsible for siring calves. Bulls can stay dominant for at least 15 years. Mating with its own offspring becomes a real possibility.

III. RHINO DNA PROFILING AND GENETIC DATABASE

The development of DNA technology has dramatically changed the identification and genetic characterization of animal species, including wildlife. A DNA profiling service is available from the Animal Improvement Institute of the Agricultural Research Council, Pretoria, South Africa, for the identification and genetic characterization of animal samples. This has now replaced blood typing in cattle (AGRICULTURAL RESEARCH COUNCIL, 2001).

The underlying principle of DNA profiling rests on the ability to analyse and compare the DNA from individual animals. Molecular genetics are used in the diagnosis of certain inheritable diseases, and the identification of individual animals. Molecular markers are used to establish the extent of diversity within species by quantifying the distance between and within breeds/populations based on differences in their genetic make-up (KOTZE *et al.*, 2002).

Individual animals can be identified by means of unique bands or microsatellites. Each animal has a unique DNA profile that distinguishes it from any other individual, even siblings or close blood relatives. The DNA markers are isolated at the molecular level which enables unique identification of individuals (KOTZE *et al.*, 2002). More important is the fact that each profile is a combination of the two parents' profiles, making it possible to construct parental lineages. This makes for a powerful tool to identify individual animals (KOTZE *et al.*, 2003).

Each animal need only be tested once in his/her lifetime after which the profile is stored in a database that is accessible for future reference. The data and knowledge build the foundation for the better use, development, maintenance and conservation of animal diversity. This technology is accepted in courts and can lead to the conviction of criminals.

Although the technology has been developed in general, a species-specific method of discriminating between the individual DNA materials is required. The MPB pioneered a Rhino Genetic Database in a joint collaborative project with the Agricultural Research Council's Animal Improvement Institute during 2001. The project cost approximately 14,000 US$ that was funded by the Rhino & Tiger Fund. It is now possible to identify every single rhino individually using blood or tissue material. The rhino database is being completed to host an individual genetic fingerprint of every single rhino on the MPB's Protected Areas.

IV. APPLICATION

The MPB has detailed information of rhino movements and habitat usage at a reserve level which has led to an improved decision-making process regarding the management of provincial populations. This was only made possible by having identifiable individuals. Each rhino has its own personal Identikit, or file, containing its individual performance and life history, and identity photographs.

Based on the DNA profile and database, parentage determination, individual identification, species identification, and the determination of the genetic structure (genetic variation, rate of inbreeding and genetic distances) between the populations can now be determined. The added advantage of the pioneering work undertaken is that samples from other organizations and from populations held by the private sector can be similarly analysed. Now that the initial cost of developing the technique has been covered, the cost for an individual sample is approximately 21 US$. The same type of approach is currently contemplated for the re-located elephant, *Loxodonta africana*, from Kruger National Park to various smaller protected areas in the country.

The main applications in conservation are the characterization of populations, within population selection, the degree of relatedness of individuals and in mating programs. The prevention of crossbreeding and the development of larger gene pools are important to sustain genetic variation (KOTZE *et al.*, 2003). The levels of inbreeding in a population, if any, can be determined. Cases or potential cases of inbreeding can be identified. Actively breeding bulls and fertile cows can be identified for use in establishment of new populations (Figure 2). In the same way surplus aged bulls or cows can be identified for trophy hunting purposes.

Each individual is now also issued with his/her own Identity certificate, containing its DNA genetic profile. In addition, it has become possible to develop a studbook for each population. This information will allow managers to monitor the genetic characteristics of individual animals. It will also enable them to determine the exact parentage of each calf to prevent future inbreeding in small populations (Table I).

The database will also prove invaluable to forensic investigators dealing with poaching cases. Together with ear-markings and implanted micro-chips this technology will ensure improved tracking of movements, serve as deterrent for poaching, and added improved forensics. This can enhance the capacity to monitor the movements of animals across provincial, national and regional borders.

The intensive monitoring and management of white rhino based on individual identification is likely to be most effective for medium-sized populations. If less than a handful of rhino occur on a single small area, it may be possible to follow this population through individual recognition of every single animal. If populations are larger than a few hundred individuals it is unlikely that individual recognition and DNA profiling can be managed from a logistical and financial point of view. The probability of inbreeding in large populations would be low.

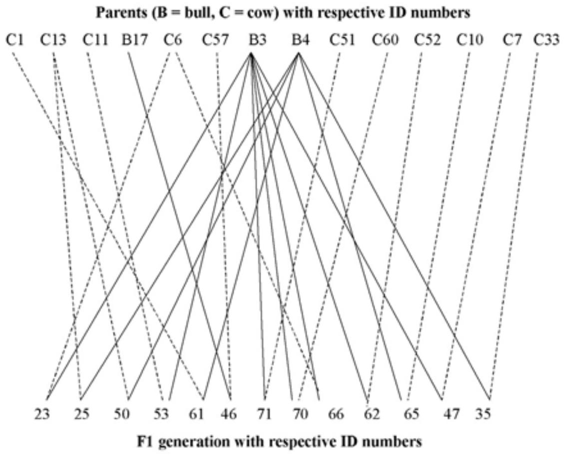

Figure 2: Linkage between white rhino, *Cerathotherium simum*, bulls and cows and F1 generation. Linkages between bulls and calves are indicated by a solid line whereas the cow-calf linkage is expressed by a dashed line. Bull 17 sired only 1 calf, whereas bulls 3 and 4 respectively sired 7 and 5 calves. Cows 13 and 6 had 2 calves during the study period. ID numbers: DNA profile identity numbers.
Figure 2 : Relations parentales entre des mâles (B) et des femelles (C) adultes de rhinocéros blanc, *Cerathotherium simum* (en haut) avec la génération F1 (jeunes numérotés, en bas). La relation entre un père et son jeune est indiquée par une ligne pleine, celle entre une mère et son jeune par une ligne tiretée. Le mâle 17 a engendré 1 jeune, tandis que les mâles 3 et 4 ont engendré respectivement 7 et 5 jeunes. Les femelles 13 et 6 ont eu 2 jeunes au cours de la période d'étude. ID numbers : numéro d'identité établi d'après les profils d'ADN.

V. CONCLUSION

Successful management depends on the ability to make decisions before problems arise. Decision-making needs to be based on reliable information. Modern technology is advancing at a rapid pace and biotechnology is no exception. It is of vital importance that all available technology is utilised for the benefit of the wildlife industry - both commercial, small-scale and conservation enterprises.

The Rhino DNA profiling and database that have been pioneered provide a powerful tool to assist with decision-making, particularly within a meta-population context.

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TABLE I
Results of the comparison of 12 different allele pairs to determine the parentage of white rhino, *Cerathotherium simum*, calf 66 that was born from cow 6 and that could have been sired by either bull 4 or 1. The positive match for all allele pairs indicates that the calf was sired by bull 1. A single mismatch is sufficient to disqualify a bull as the potential father.

TABLEAU I
Résultats de la comparaison de 12 paires d'allèles pour déterminer la parenté du jeune rhinocéros blanc, *Cerathotherium simum*, n° 66 né de la femelle adulte n° 6 et qui a pu avoir eu pour père le mâle adulte 4 ou le mâle adulte 1. L'adéquation positive (match = 1) de tous les allèles montre que le jeune a été engendré par le mâle 1. Une seule inadéquation (mismatch = 0) suffit pour disqualifier un mâle comme père potentiel.

Locus	Allele comparison between Calf 66, Cow 6 and Bull 4				Allele comparison between Calf 66, Cow 6 and Bull 1			
	Bull n° 4	Cow n° 6	Calf n° 66	Match (1)/ Mismatch (0)	Bull n° 1	Cow n° 6	Calf n° 66	Match (1) Mismatch (0)
DB42	330 330	324 326	324 326	0	326 326	324 326	324 326	1
BR4	109 109	109 109	109 109	1	109 109	109 109	109 109	1
RHI32A	234 234	234 248	248 248	0	234 248	234 248	248 248	1
BR6	133 153	133 133	133 135	0	133 135	133 133	133 135	1
DB52	217 221	219 219	217 219	1	217 219	219 219	217 219	1
DB66	201 203	203 203	201 203	1	210 201	203 203	201 203	1
RHI7C	253 255	255 255	255 255	1	255 255	255 255	255 255	1
DB23	170 170	170 170	170 170	1	170 170	170 170	170 170	1
DB14	273 273	273 273	273 273	1	273 273	273 273	273 273	1
DB1	127 127	129 129	129 129	0	129 129	129 129	129 129	1
BR17	118 118	118 118	118 118	1	118 118	118 118	118 118	1
DB44	173 181	181 181	181 181	1	181 181	181 181	181 181	1
Outcome:	Calf of cow 6 NOT sired by bull 4			0	Calf of cow 6 sired by bull 1			1

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UTILISATION DE LA TECHNOLOGIE DE L'ADN POUR L'IDENTIFICATION DU RHINOCÉROS BLANC (*CERATHOTHERIUM SIMUM*) ET SON APPLICATION À LA CONSERVATION

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MOTS-CLÉS : Rhinocéros blanc, *Cerathotherium simum*, gestion, technologie de l'ADN, identification génétique, aide à la décision, Mpumalanga, Afrique du Sud.

RÉSUMÉ

L'Afrique du Sud joue un rôle critique au niveau international pour la conservation du rhinocéros blanc, Cerathotherium simum*. Le Bureau des Parcs de Mpumalanga (BPM) est l'organisme légal en charge de la conservation. Certaines de ses aires protégées renferment des populations-clés de rhinocéros blancs. Aucune de ces population n'est assez grande pour être viable seule. Une augmentation des effectifs est limitée par le manque d'habitats disponibles et par la compétition intra-spécifique. Pour rompre ces limites et éviter la consanguinité il faut mettre en œuvre une stratégie basée sur le concept de méta-population. L'identification et la reconnaissance permanente des individus de rhinocéros sont nécessaires pour une gestion efficace des petites sous-populations de la méta-population. Cela a été réalisé dans un passé récent grâce au micro-écornage, à l'entaille des oreilles et à la compilation de fichiers individuels. Cependant, malgré une documentation et un suivi intensifs de ces sous-populations, il n'a pas été possible d'établir avec certitude dans quelle mesure les mâles adultes étaient responsables des générations successives de jeunes. Pour cela des données génétiques sont nécessaires. La technique de l'établissement du*

profil d'ADN basée sur la génétique moléculaire a été développée par Institut d'Amélioration des Animaux du Conseil de la Recherche Agricole, en utilisant le matériel récolté sur des rhinocéros blancs des aires protégées du BPM. Les individus peuvent maintenant être identifiés sans erreur à l'aide des bandes uniques des micro-satellites. Chaque profil individuel est caractérisé par une combinaison des deux profils des parents, ce qui permet de déterminer les relations parentales. Une base de données génétiques des rhinocéros des aires du BPM a pu ainsi être établie. L'information procurée par cette base est donc utilisée pour déterminer la parenté, la productivité animale, la variation génétique, le taux de consanguinité et les distances génétiques entre populations. La technique du profil d'ADN est un outil puissant à la disposition du BPM pour pallier les conséquences génétiquement nuisibles de l'existence de populations petites et isolées. On peut maintenant décider quel individu peut être déplacé d'une sous-population à une autre [pour rompre l'isolement].