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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