RHINOCEROS GENETICS: THE STATE-OF-THE-ART AND APPLICATION TO CONSERVATION MEASURES

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The mammalian family Rhinocerotidae, is one of three families that comprise the Order Perissodactyla. There are five extant species of rhinoceros (Table 1). All five species are listed as endangered in the 1986 IUCN Red List of Threatened Animals (IUCN, 1986). Estimated numbers of African rhinos have been declining rapidly, due mainly to their selective removal from available habitat by poaching (Bradley Martin, 1982). Of the three Asian species, population numbers are stabilized only for the Indian rhinoceros. Some 1987 estimates for population numbers of extant rhino species are also listed in Table 1.

Decline in population numbers and increasing isolation and fragmentation of rhino populations raises concern for the long-term survival of this unique group of mammals, even if population numbers are stabilized at current levels, because small isolated populations are vulnerable to extinction from random demographic events, inbreeding, and genetic drift.

Current strategy for conservation of rhinoceroses is in the context of national plans with oversight by the IUCN/SSC African Elephant and Rhino Specialist Group (AERSG), IUCN/SSC Rhino Specialist Group, United Nations Environmental Program, UNESCO, and other international and national agencies. In some cases populations of rhinos within a single nation-state are so low that their long-term viability is seriously in question. Under these circumstances, current conservation strategies for rhinos involve proposals for genetic mixing of some of the named subspecies. Three of the extant rhino species have named subspecies (Table 1).

The use of subspecies designations in the zoological nomenclature was established long before modern studies in population genetics revealed spatial and temporal patterns of genetic diversity within species of mammals. Often, subspecies status is conferred assuming that it reflects genetic and/or ecological differences. However, the results of modern genetic studies employing chromosomal analysis, protein electrophoresis, and other biochemical-genetic methods have not always been consistently correlated with recognized subspecies designations. Subspecies were traditionally designated by morphological criteria including minor cranial and pelage differences. Often these were not subjected to the types of statistical analysis that are available today. Consequently, the subspecific distinctions among mammals are somewhat arbitrary and inconclusive, particularly among neighboring subspecies with contiguous distribution or those showing continuous variation.

Alternately, populations designated as only being distinct at the subspecies level have been shown to be reproductively isolated. In some instances, chromosomal differences between subspecies have been shown to be of sufficient magnitude that progeny of first-generation crosses between subspecies are sterile.

Comparative genetic studies may be useful in providing data that will help in the evaluation of the degree of evolutionary differentiation of rhino populations, subspecies and species. Previous genetic studies of rhinoceroses have been limited to investigations of chromosome numbers for relatively few individuals of a limited number of populations of a few named subspecies.

Thus, it is recognized that additional genetic studies of rhinoceroses are urgently needed. The purpose of this paper is to provide a review of the data gathered in our laboratory in San Diego or in collaboration with investigators elsewhere.

BLACK RHINOCEROS

Limited chromosomal data has been published on black rhinoceroses. An adult female specimen from Kenya was studied by Hungerford and Snyder (1967). Heinchen (1969) reported that an animal from Krueger Park had 84 chromosomes. To our knowledge, no other geographic forms of black rhinoceros have been subjected to chromosomal investigations.

We have studied the chromosomes of 16 individual black rhinos for which we are reasonably certain of the subspecies status of 13. Of these, with the help of the black rhino SSP species coordinator, Ed Maruska, we have been able to determine that 12 are Diceros bicornis michaeli. All of these individuals possess 84 chromosomes. However, we have found a variation in the number of chromosome arms in individuals of this subspecies. C-banding reveals that this variation is due to the presence or absence of heterochromatic small arms on chromosomes exhibiting G-banding homology.

To date, we have studied one male individual held in Los Angeles that belongs to the D. b. minor subspecies. Remarkably, this individual has a smaller number of chromosome arms than the michaeli individuals we have studied. C-banding analysis of this single male animal reveals only four chromosomes that have heterochromatic small arms of appreciable size. In this regard, the pattern of heterochromatin in this single michaeli individual is more similar to that of white rhinos, Ceratotherium simum.

Major karyotypic variation in the context of variable numbers of acrocentric chromosomes has been observed in other mammals, e.g., Peromyscus (Pathak et al., 1973).

Further chromosomal studies of black rhino subspecies should be conducted in order to learn more about the chromosomal differentiation of the geographically distributed remnant populations of this endangered species.

SUMATRAN RHINOCEROS

The Sumatran rhinoceros, Dicerorhinus sumatrensis, is an endangered species for which efforts are underway to establish captive populations derived from animals captured in habitats designated for deforestation and agricultural purposes.

As a result of this effort, a total of ten Sumatran rhinos are now in

captivity in Indonesia, Malaysia, Thailand, and Great Britain. At the time of writing, potential breeding pairs exist in the Jakarta Zoo and at a capture site in Sumatra. In order to constitute additional breeding pairs, animal translocations will be made producing pairs of animals from different subspecific backgrounds. While the establishment of breeding groups is of the highest priority, some concern does exist as to whether the pairing for reproduction of individuals from different geographic regions is appropriate. The potential consequences of inappropriate pairing for reproduction of these animals include a reduced rate of population growth, the production of offspring with reduced fertility, and the production of individuals with genetic backgrounds that do not accurately reflect the situation found in wild populations. In recent times the species has occurred on Borneo (D. s. harrissoni), Sumatra and Malaysia (D. s. sumatrensis), and on the Asiatic mainland as far north as Assam (D. s. lasiotis) (Groves and Kurt, 1972). In the Mammalian Species account for the Sumatran rhino, Groves and Kurt summarized the genetic knowledge of this species in the following way: "Nothing whatever is known of the genetics of this species."

When a female Sumatran rhinoceros died unexpectedly at the Port Lympne Estate in Kent, England, zoo director Dr. Tom Begg collected skin biopsy specimens that were forwarded to our laboratory in San Diego. Cell cultures were successfully established and chromosomal preparations made. The female, "Subur," possessed 82 chromosomes. With the exception of the sex chromosomes, we believe the chromosomal complement consists entirely of acrocentric chromosomes. The sex chromosomes are submetacentric with prominent distal blocks of heterochromatin. This individual was captured on Sumatra and, accordingly, would belong to the sumatrensis subspecies. It is anticipated that opportunities for sample collection will arise during the process of translocating animals in order to create breeding groups. Samples will be collected by individuals involved in the field activities of the AAZPA Sumatran Rhino Trust and forwarded to San Diego for analysis.

WHITE RHINOCEROS

Two subspecies of white rhino, Ceratotherium simum, are recognized. Unlike the black rhino that, until recently, consisted of contiguously-distributed populations, the white rhino is thought to have been discretely distributed for thousands of years (Groves, 1972), although this is not a unanimous opinion (D. Western, pers. comm.). The Southern form, C. s. simum, went through a population reduction and bottleneck estimated to be approximately 30 animals within the last 100 years. The previously more numerous Northern form, C. s. cottoni, survives now as a single population estimated at 17 - 20 animals in Garamba National Park in Zaire. A captive population of Northern white rhinos is held in the Dvur Kralove Zoo in Czechoslovakia.

Chromosomal studies of Southern white rhinos in Kruger National Park involved direct preparations from bone marrow. These studies were successful on only a few numbers of individuals, but, when successful, a diploid chromosome number of 82 was obtained. More recent studies involving cell culture obtained diploid chromosome numbers of 84 utilizing statistical analysis of a large number of well-prepared metaphase plates.

We have studied the chromosomes of nine Southern white rhinos, three Northern white rhinos and one first-generation hybrid between parents belonging to the two different subspecies. Successful blood cultures always revealed a diploid chromosome number of 82. Early passaged fibroblasts revealed a diploid number of 82 as well. However, upon extended culturing, diploid chromosome numbers of 84 and higher have been obtained. We currently believe that, with extended time in culture, artifactual cell transformation occurs resulting in chromosome counts of varying numbers including tetraploidy.

The availability of a first-generation captive-born individual, one of whose parents was a Northern white rhino and one a Southern white rhino, provides the opportunity for detailed comparisons of the chromosomes of the two subspecies in a single individual. We can conclude at this time that the diploid chromosome number for both C. s. simum and C. s. cottoni is 82 and that, in broad perspective, the G-banding patterns of their chromosomes are highly similar if not identical.

An electrophoretic comparison of enzymes and other blood proteins of the two white rhino subspecies, involving an analysis of 31 electrophoretic loci resulted in a very small intraspecific distance between the two living white rhino subspecies (Merenlender, A., Woodruff, D. and Ryder, O.A., in preparation). A study involving comparison of mitochondrial DNA from one Northern white rhino and two Southern white rhino individuals suggested that the mitochondrial DNAs of the two rhinos differ by approximately 4% in their nucleotide sequences (George, M., Puentes, L.A. and Ryder, O.A., 1982). By comparison to calibrations made for primate species, these results indicated that the white rhino subspecies last shared a common ancestor at least two million years ago (George, M., Puentes, L.A. and Ryder, O.A., 1982). These results, while not necessarily in conflict, indicate that further analyses are necessary in order to provide a more complete picture about the genetic differentiation of the two named subspecies of Ceratotherium simum.

INDIAN RHINO

The greater Indian rhinoceros has one named subspecies. Currently, two populations exist in the wild, one in India in Assam and the other in Nepal. We have studied the chromosomes of a single male Indian rhino and have determined a chromosome number of 82. This is consistent with a previous report in the literature (Wurster, D.H. and Benirschke, K., 1968). We have obtained G- and C-banded preparations from the single individual and hope to analyze additional samples.

CONCLUSIONS

Additional genetic studies of all extant rhino taxa are clearly indicated with priority allocated to investigations of of black and Sumatran rhinos. Chromosomal analysis has been shown to be an important aspect of the genetic comparisons following the findings derived from the single animal of Zimbabwe origin held in the Los Angeles Zoo. Additional samples urgently need to be collected for chromosomal, electrophoretic and mitochondrial DNA analyses. At the SSP/AERSG workshop held October, 1986 in Cincinnati, OH, protocols were

developed for sample collection by Dr. Eric Miller of the St. Louis Zoo. Genetic studies of rhinos are currently being undertaken by our group at the San Diego Zoo and in the laboratory of Dr. Don Melnick, Department of Anthropology, Columbia University, NY. The findings of these continuing investigations may significantly impact conservation management plans for the endangered rhinoceroses and, for this reason alone should be expedited.

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REFERENCES

Bradley Martin, E. and Bradley Martin, C., 1982. Run Rhino Run. Chatto and Windus. London.

George, M., Puentes, L.A. and Ryder, O.A., 1982. Genetic differences between subspecies of white rhinoceroses (in German). International Studbook of African Rhinos. Berlin Zoological Garden. Berlin, German Federal Republic.

Groves, C.P., 1972. Ceratotherium simum. Mamm. Sp. 8: 1-6.

Groves, C.P. and Kurt, F., 1972. Dicerorhinus sumatrensis. Mamm. Sp. 21: 1-6.

Heinlchen, I.G., 1969. Karyological studies on South African perissodactyla. J. So. Afr. Vet. Med. Assoc. 40: 99-100.

Hungerford, D.A. and Snyder, R.L., 1967. Somatic chromosomes of a black rhinoceros (Diceros bicornis, Gray, 1821). Am. Nat. 101: 357-358.

Hsu, T.C. and Benirschke, K., 1973. Ceratotherium simum. Atlas of Mammalian Chromosomes, Vol. 7, Folio 339.

IUCN, 1986. 1986 IUCN Red List of Threatened Animals. IUCN/CMU. Cambridge, England.

Pathak, S., Hsu, T.C. and Arrighi, F.C., 1973. Chromosomes of Peromyscus (Rodentia, Cricetidae). IV. The role of heterochromatin in karyotypic evolution. Cytogenet. Cell Genet. 12: 315-326.

Wurster, D.H. and Benirschke, K., 1968. The chromosomes of the great Indian rhinoceros (Rhinoceros unicornis, L.). Experentia 24: 511.