Mitochondrial DNA Variability and Conservation Genetics of the Sumatran Rhinoceros

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Introduction

Among mammalian species cited as endangered by the World Conservation Union (IUCN), the United States Department of the Interior (USDI), and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the Sumatran rhinoceros (*Dicerorhinus sumatrensis*) is one of the most threatened. Surviving populations are dwindling rapidly due to heavy poaching and habitat conversion (Fig. 1). On Sumatra alone the rhinoceros population has declined by more than half from 420–875 to 235–320 individuals over the past decade (Captive Breeding Specialist Group Species Survival Commission of the IUCN 1993). The number of Sumatran rhinoceros surviving today is between 413 and 563, and captive breeding programs established to contribute to the survival of this species have been unsuccessful (Foose & van Strien 1995).

Managed breeding sanctuaries in natural habitats are being proposed as an alternative to captive breeding and as a refuge for rescued animals (Foose & van Strien 1995). These units are intended to concentrate large numbers of animals in protected areas. However, given all the conservation efforts being carried out on behalf of the Sumatran rhinoceros (Khan 1989; Captive Breeding Specialist Group 1994) and the recent discussions surrounding these efforts (Rabinowitz 1995*a*, 1995*b*; Andau 1995; Hutchins 1995; Foose et al. 1995; Sumardja 1995), genetic information on this species is strikingly sparse. We know little about the extent of genetic variability in remnant populations or the extent of genetic divergence among them. The little we do know is based on the slowly evolving mtDNA ribosomal genes, and thus is not adequate for conservation management of conspecific populations (Amato 1994; Morales & Melnick 1994).

The purpose of our study was to survey the pattern and extent of genetic heterogeneity among the remaining populations of Sumatran rhinoceros by analyzing the rapidly evolving mitochondrial control region. Our results constitute the most extensive contribution so far to our understanding of the genetic structure of the Sumatran rhino. These data, together with additional data from nuclear genes, will provide a genetic foundation that combined with other non-genetic data will further enhance the prospects of recovery of this highly endangered mammal.

Methods

Fifteen wildborn Sumatran rhinoceros were included in our study, representing four populations (Fig. 1) in the three major areas of distribution for this species: Malay Peninsula (four individuals, studbook No. 1, 12, 13, 19); Sabah in Borneo (six individuals studbook No. 17, 26, 31, 36, 38, 40); Riau in Sumatra (three individuals, studbook No. 18, 24, 25); and Bengkulu in Sumatra (two individuals, studbook No. 33, 34). Samples were obtained as blood or freshly plucked hairs (CITES permit no. US781482). Total genomic DNA was obtained using standard phenol/chloroform extraction (Maniatis et al. 1982) and ethanol precipitated. The control region of the rhinoceros mtDNA was amplified with a modified version of the universal primers developed by Kocher et al. (1989) as follows: L15926 5′-TACACTGGTCTTGTAAACC-3′ and H00651 5′-AAGGCTAGGACCAAACCT-3′.

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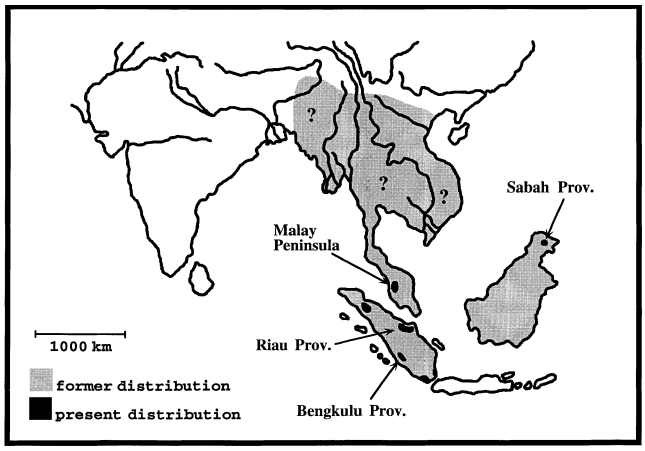

Figure 1. Distribution map of the Sumatran rhinoceros, including the historical range, the remaining populations, and the location of sampling sites indicated by arrows. Question marks correspond to areas where Sumatran rhinos may still exist.

The resulting amplified product was digested with 22 restriction endonucleases: Acc I, Aci I, Ase I, Bsm I, BsmF I, Bsp1286 I, BstN I, BstU I, Dde I, Dpn II, Dra I, Fok I, Hae III, Hha I, Hinf I, Hpa II, Hph I, Nla III, Rsa I, Sau96 I, Ssp I, and Taq I. Fragment patterns were resolved in 1.5% to 2% agarose gels stained with ethidium bromide. Restriction sites were mapped onto the control region using the partial endonuclease digestion mapping procedure (Morales et al. 1993; Morales & Melnick 1994).

Distance values between haplotypes (i.e., substitutions per nucleotide site) were obtained according to Nei and Tajima (1981) using the REAP program (McElroy et al. 1991). Character based cladistic analysis of haplotype relationships was performed with the program PAUP Version 3.1.1, using the exhaustive search procedure (Swofford 1993). Because the likelihood of a site loss is much greater than a site gain, we used Dollo parsimony (DeBry & Slade 1985). A bootstrap analysis with 2000 replicates was performed, and a 50% majority-rule bootstrap consensus tree was generated.

Within population variation was estimated using the nucleotide diversity (π) index of Nei and Tajima (1981). Nucleotide divergence values (d_A) among populations were estimated according to Nei and Li (1979) using the program REAP (McElroy et al. 1991). In this procedure total nucleotide diversity is estimated, and the component of this diversity not explained by within population variation is extracted.

Results

Four different Sumatran rhino D-loop haplotypes were found in a 1550-bp long segment ($tRNA_P$ to $tRNA_F$). One from the Malay Peninsula and the Riau Province in Sumatra (studbook No. 1, 12, 13, 19, 24, and 25), a second from the Riau Province (studbook No. 18), a third from Bengkulu Province in Sumatra (studbook No. 33 and 34), and a fourth from the Sabah Province in Borneo (studbook No. 17, 26, 31, 36, 38, and 40). Two African rhinoceros haplotypes were included as outgroups. A total of 66 sites was identified for all haplotypes surveyed, representing 275 bp interspersed throughout the fragment. Among the Sumatran rhinoceros, five restriction sites distinguish the four haplotypes found as presented in Table 1. Total number of character differences among all haplotypes surveyed are presented in Table 2. A complete binary matrix of the restriction site data is available from JCM upon request.

Estimates of substitution differences per nucleotide site among all haplotypes are presented in Table 2. These divergence values range from 0.27% between The Malay Peninsula and one haplotype from Riau Province in Sumatra, to 13.3% between the Sumatran haplotype from Riau and the white rhinoceros. Among the Sumatran rhinoceros the average divergence among Malay and Sumatran populations is only 0.3%, whereas the average difference among western populations and Borneo is 1.0%. These larger values reflect three fixed differences between Borneo and the other regions, two of them being synapomorphies.

The exhaustive search procedure resulted in one most parsimonious tree (length = 47) with a consistency index of 0.957 (Fig. 2). Eight unambiguous synapomorphies separate the Sumatran rhinoceros clade from the other two African forms. Within the Sumatran rhinoceros, two synapomorphies separate the Malay Peninsula-Sumatra clade from the Sabah, Borneo clade. This split corresponds with the recognition of two separate subspecies (Groves 1965).

Table 1. Restriction site differences between Sumatran rhino haplotypes.*

	HinfI(360)	*RsaI(420)*	*NlaIII(440)*	*DpnII(510)*	*BsmFI(850)*
Malay-Riau	1	1	1	1	1
Riau (No. 18)	1	0	1	1	1
Bengkulu	1	1	1	1	0
Sabah	0	1	0	0	1

**Number in parentheses corresponds to the relative position of the restriction site with respect to the labeled primer b999. A "1" denotes the presence of the site, and a "0" denotes its absence.*

Table 2. Total number of character differences among haplotypes (above diagonal) and estimates of evolutionary distance among haplotypes as per Nei and Tajima (1981) (below diagonal).

	Malay-Riau	*Riau (No. 18)*	*Bengkulu*	*Sabah*	*white*	*black*
Malay-Riau	0.0000	1	1	3	31	28
Riau (No. 18)	0.0027	0.0000	2	4	32	27
Bengkulu	0.0029	0.0056	0.0000	4	30	27
Sabah	0.0084	0.0114	0.0115	0.0000	30	27
white	0.1268	0.1327	0.1239	0.1277	0.0000	25
black	0.1106	0.1084	0.1054	0.1109	0.1063	0.0000

Nucleotide divergence values among Sumatran rhinoceros populations are summarized in Table 3. These data show no genetic distinction among the samples from The Malay Peninsula and eastern Sumatra (Riau Prov.); slight genetic differentiation between western (Bengkulu Prov.) and eastern Sumatran populations; and larger genetic distinction between the Bornean rhinoceros and the rest of the samples. Whether one examines the phylogenetic relationships of haplotypes or populations, the same patterns emerge. Figure 2, a cladistic analysis of informative restriction sites, can be taken as representative of the results obtained in this study. The consistency of results across methods reflects the strong phylogenetic signal in the data despite the small number of differences.

Discussion

In most cases the Sumatran rhino populations surveyed are fixed for a single mtDNA haplotype, and regardless of how minimal the divergence estimates are among haplotypes (Table 1), these fixed differences reflect a marked extant population structure and are consistent with a known biogeographical history of separation (Moritz 1994) (Tables 2 & 3). The islands of Sumatra and Borneo and the Malay Peninsula have been isolated by water for at least 10,000 years (Heaney 1984), but it is likely that the rhino populations have been isolated from each other for a much longer period. During glacial maxima, when most of the Sunda shelf was above sea level, populations on the eastern and western sides of the shelf may have been separated by a long semiarid corridor crossed by a series of river basins that extended north to south, effectively separating suitable Sumatran rhino habitat (Morley & Fenley 1987). This scenario may explain the mtDNA distribution we found and is consistent with current taxonomic designations that recognize the Borneo population as a different subspecies (Groves 1965). Individuals from the Malay Peninsula and east Sumatra were apparently not separated by climatic conditions during the recurring Pleistocene events, which is again reflected in patterns of mtDNA difference. Interestingly, within present day Sumatra, east and west populations of rhinoceros may have had more restricted gene flow than expected because of the presence of the Barisan Mountains. This range consists of a volcanic arc (the Cainozoic volcanic arc) that extends north to south in western Sumatra, with activity recorded as far back as the early Cretaceous period (Hutchinson 1989). The east Sumatra group is virtually indistinguishable from the Malay Peninsula population, whereas the west coast group from the Kerinci-Seblat National Park shows some difference.

Because of failure of captive breeding programs (Foose & van Strien 1995), rapid disappearance of suitable habitat, and heavy poaching, creation of wild "sanctuaries" for the Sumatran rhino is being proposed. These will be established in semi-natural conditions, both with native and rescued animals. The success of the sanctuary program will depend in part on an accurate monitoring of

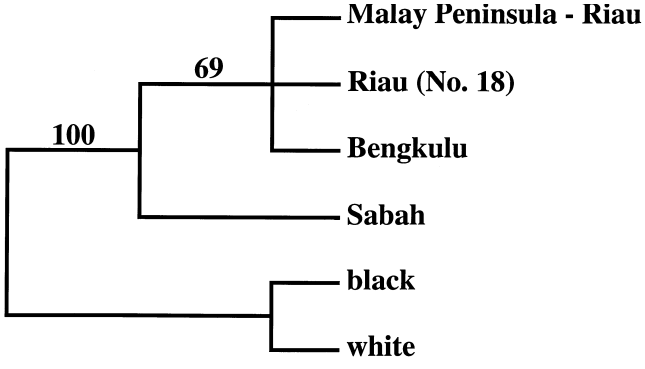

Figure 2. Cladistic relationships among haplotypes using the method of parsimony (PAUP) with the bootstrap "consensus" values above the branches.

Table 3. Nucleotide divergence estimates (d_A) among Sumatran rhinoceros populations.*

	Malay Peninsula	*Riau Prov.*	*Bengkulu Prov.*	*Sabah Prov.*
Malay Peninsula	0.0000			
Riau Prov.	0.0000	0.0018		
Bengkulu Prov.	0.0029	0.0029	0.0000	
Sabah Prov.	0.0084	0.0085	0.0115	0.000

**Values at the diagonal represent estimates of nucleotide diversity (π) within populations.*

population genetic parameters in this new metapopulation. We have identified at most three management units (Moritz 1994) among the remaining populations of Sumatran rhinoceros: Malay Peninsula-East Sumatra (PM/ES), West Sumatra (WS), and Borneo (B). The sanctuaries will likely bring together individuals from different populations within and possibly between these different management units. Because of the extremely low levels of genetic divergence and the absence of fixed haplotype differences among the PM/ES and WS management units (Tables 1 & 2; Fig. 2), we think these might represent populations that have been exchanging migrants until very recently. Therefore, interbreeding individuals within and among these units might not have serious negative genetic consequences. However, our data are preliminary, do not include an examination of the nuclear genome, and therefore close attention must be paid to the fitness and genetic makeup of inter-crossed progeny. We believe this is a safe and responsible step to take in the important task of saving this critically endangered species. Nevertheless, it is vital to keep in mind that as important as the infusion of "fresh" genetic variation into inbred lines is, it is equally important to avoid disrupting coadapted gene complexes that might result in outbreeding depression (Lynch 1991).

The Sumatran rhinoceros from Borneo constitutes a special case that should be carefully considered before major management decisions are made. Because of the larger genetic divergence from other Sumatran rhinoceros populations (Tables 1 & 2; Fig. 2) and their characteristic morphological phenotype that has merited a separate taxonomic distinction, the ideal situation would be to maintain this lineage as a separate management unit. Unfortunately, because the remaining natural populations of the Sabah Sumatran rhinoceros are estimated to be 50 to 100 individuals (Foose & van Strien 1995; Andau, unpublished data), we might not have the luxury of managing this group as an entirely independent unit. However, before moving ahead to cross Sumatran rhinoceros from Borneo with those from The Malay Peninsula and Sumatra, we should examine closely the example of the northern white rhinoceros (*Ceratotherium simum*) of Garamba National Park, Zaire. In this case considerable success was achieved from the intense protection of an even smaller remnant population, reporting a 100% increase in numbers in just one decade (Foose 1995). It may be that if the remaining Sabah rhinoceros can be brought into an area of sufficient size and habitat quality and can be protected from poachers, they will recover demographically. If this is not possible, the challenge in the near future for this and the other Sumatran rhinoceros management units will be to develop strategies to maintain a metapopulation structure that allows enough gene flow to avoid inbreeding depression without compromising the fitness and phylogenetic integrity of the animals within each unit.

We believe there are potentially at most three management units among the remaining Sumatran rhinoceros. The two units in the western part of the species range (PM/ES and WS) can be effectively combined into one unit, if this is demographically warranted, without serious negative genetic consequences. We do, however, believe that the eastern or Borneo management unit should if at all possible be kept separate. Short of this, efforts should be made to minimize the disruptive and potentially fitness reducing consequences of gene flow from long separated, genetically distinct conspecifics.

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