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RESEARCH ARTICLES

Assessment of Conservation Units for the Sumatran Rhinoceros (*Dicerorhinus sumatrensis*)

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An assessment of conservation units for the Sumatran rhinoceros (*Dicerorhinus sumatrensis*) was conducted using a population aggregation analysis (PAA) of mitochondrial DNA site substitutions. Populations were defined as the three geographically separated regions of West Malaysia, Sumatra, and Borneo. The intent of this assessment was to explore management options for this highly endangered lineage rather than conduct a traditional taxonomic revision.

Individual DNA positions were not diagnostic for any population. A single haplotype provided a character as support for diagnosing the West Malaysian and Bornean population. The haplotypes on West Malaysia and Sumatra were more similar to each other than either was to the one on Borneo. These data, and a review of the morphological characters, support the option of treating Sumatran rhinos as a single conservation unit, providing managers with greater flexibility in managing the unique *Dicerorhine* lineage. © 1995 Wiley-Liss, Inc.

Key words: subspecies, population aggregation analysis, mitochondrial DNA, conservation unit

INTRODUCTION

The Sumatran rhinoceros (*Dicerorhinus sumatrensis*) is a highly endangered species currently confined to a few remnant upland forest areas in Peninsular Malaysia, Sumatra, and Borneo. Like the other extant rhinos, the Sumatran rhino originally had an extensive distribution. Until the beginning of this century it ranged from India (Assam and Bengal) through Myanmar, Thailand, Cambodia, Laos, Viet Nam, China, Malaysia, and Indonesia (Sumatra and Kalimantan) [Groves, 1983; and Borner, 1979].

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While historically Sumatran rhinos used habitats that included lowland forests and natural clearings, their presence in upland forest and mountainous regions explains why the species has persisted in more areas and in larger numbers than the historically sympatric Javan rhino (*Rhinoceros sondaicus*), which is confined to lowland forests [Santiapillai and MacKinnon, 1993; Penny, 1988; and Van Strein, 1986]. These mountainous areas are the last to be deforested and the most difficult in which to hunt the surprisingly nimble animal [Santiapillai and MacKinnon, 1993; Khan et al., 1993]. Sumatran rhino tracks have been found up to 2,000 m in elevation.

Currently, the only potentially viable populations persist in Sumatra (Gunung Leuser National Park, Torgamba Forest, Kerinci-Seblat National Park, Barisan Selatan National Park, and Gunung Patah), in West Malaysia (Taman Negara and Endau Rompin), and in Kalimantan (Danum Valley Conservation Area, Tabin Wildlife Reserve, proposed Lower Kinabatangan Wildlife Reserve, and possibly the proposed Pulong Tau National Park). However, the total number of animals is probably under 400 (including 24 animals in captive breeding programs). In addition to deforestation, the rhinos are threatened by commercial hunting for their horn in both protected and nonprotected areas. In 1990, at least ten rhinos were poached in Kerinci-Seblat National Park in Sumatra [Santiapillai and MacKinnon, 1993]. An organized conservation program is essential to the survival of this species.

The governments of Indonesia and Malaysia, as well as international conservation organizations (The Wildlife Conservation Society, The World Wide Fund for Nature, IUCN Captive Breeding Specialist Group, and The Sumatran Rhino Trust), have mounted a major effort to conserve this species. Management plans include research, greater protection of wild populations, and a controversial captive breeding program. Since management strategies may include translocating animals or gametes, the question of conservation units is of great importance.

Groves [1967] divides the species into three subspecies (*D.s. sumatrensis* [Sumatra and Malaysia], *D.s. harrissoni* [Borneo], and *D.s. lasiotis* [Myanmar and India]) based on measurements of eight morphological characters. While there have been recent reports of rhinos in the Naga Hills area of Northern Myanmar [Rabinowitz and Schaller, personal communication], at this time the status of these populations is unknown. For conservation management purposes, we have investigated the three surveyed, geographically separated populations of West Malaysia, Sumatra, and Borneo, even though Groves [1967] groups Sumatra and West Malaysia together.

MATERIALS AND METHODS

Seventeen Sumatran rhinos representing the three populations (Table 1) were sequenced for 953 bases of 12S and 16S mitochondrial sequences. Individuals were sampled in a variety of manners as dictated by specific circumstances in the field and international collections. Samples included frozen blood, frozen tissue, blood preserved in RT buffer (100 mM Tris, 100 mM EDTA, and 2% SDS) and stored at room temperature, and shed hair and skin kept dry and at room temperature. All samples were obtained without harm to the study animals. Total genomic DNA was isolated for all of the blood samples by previously described standard phenol/chloroform isolation procedures [Caccone et al., 1987]. A method employing a chelating resin (Chelex 100® BioRad) optimized for forensics samples [Walsh et al., 1991] was used to isolate DNA from the shed hair and skin samples.

TABLE 1. Sumatran rhinoceros samples included in this study

International studbook number	Location
Dicerorhinus sumatrensis	
6	Sumatra
22	Sumatra
24	Sumatra
27	Sumatra
28	Sumatra
33	Sumatra
17	Borneo
26	Borneo
31	Borneo
38	Borneo
1	West Malaysia
7	West Malaysia
13	West Malaysia
15	West Malaysia
19	West Malaysia
20	West Malaysia
23	West Malaysia

Fragments of the 12S and 16S ribosomal mitochondrial genes were PCR amplified with modified universal vertebrate primers [Kocher et al., 1989]. PCR reactions were carried out in 100 μl reaction volumes with reagents from Perkin-Elmer Cetus Gene Amp Kit. Reactions were performed in a Perkin-Elmer Cetus DNA Thermal Cycler with approximately 250 ng of template DNA and a magnesium concentration of 1.5 mM. Cycling conditions were 94°C for 1 min, 55°C for 1.5 min, and 72°C for 2 min for 40 cycles. Most often, unbalanced primers were used to accomplish asymmetric PCR [Gyllensten and Erlich, 1988]. Single-stranded PCR products were cleaned and concentrated with centricon-30 columns (Amicon, Beverly, MA) and directly sequenced by the dideoxy method with reagents and protocol from USB's (Cleveland, OH) Sequenase 2.0 sequencing kit [Gatesy and Amato, 1992]. Some sequences were obtained using an automated sequencer (model 373A, Applied Biosystems, Foster City, CA) following the manufacturer's protocols. Both strands were sequenced to assure accuracy.

Sequences were assigned to local populations defined by geographical location (i.e., West Malaysia, Sumatra, and Borneo) (Table 2). Base substitutions were assessed as either characters or traits as defined by Davis and Nixon [1992]. This method, population aggregation analysis (PAA), involves successive searches for fixed differences among aggregations of local populations. Characters are attributes that are not polymorphic and are unique within populations. Traits are attributes that may be polymorphic or are not unique to a population. An assessment of conservation units for Sumatran rhinoceros was considered in light of the population aggregation analysis.

RESULTS

Four haplotypes were identified from the 17 Sumatran rhinos sampled. Only one haplotype was found in the samples from Borneo and one from West Malaysia,

TABLE 2. 12S and 16S mitochondrial DNA sequences*

SumRhinoS12S						
1	GCTTAGCCCT	AAACCTAAAT	GATTTCCCCC	AACAAAATCA	TTCGCCAGAG	TACTACTAGC
61	AATAGCCTAA	AACTCAAAGG	ACTTGGCGGT	GCTTTATATC	CCCCTAGAGG	AGCCTGTTCC
121	ATAACCGATA	AACCCCGATA	AACCTTACCA	ACCCTTGCTA	ATTCAGCCTA	TATACCGCCA
181	TCTTCAGCAA	ACCCTAAAAA	AGGAACTAAA	GTAAGCACAA	GTATAAGACA	TAAAAACGTT
241	AGGTCAAGGT	GTAGCTTATG	GGATGGAGAG	AAATGGGCTA	CATTTTCTAC	TACAAGAACA
301	ACAATTATCC	AAACGAAAGC	CCCCATGAAA	CTAAGGGCTA	AAGGAGGATT	TAGCAGTAAA
361	TTAAGAACAG	AGAGCTTAAT	TGAACAAGGC	CATAAAGCAC	GC	
SumRhinoS*12S						
1	GCTTAGCCCT	AAACCTAAAT	GATTTCCCCC	AACAAAATCA	TTCGCCAGAG	TACTACTAGC
61	AATAGCCTAA	AACTCAAAGG	ACTTGGCGGT	GCTTTATATC	CCCCTAGAGG	AGCCTGTTCC
121	ATAACCGATA	AACCCCGATA	AACCTTACCA	ACCCTTGCTA	ATTCAGCCTA	TATACCGCCA
181	TCTTCAGCAA	ACCCTAAAAA	AGGAACTAAA	GTAAGCACAA	GTATAAGACA	TAAAAACGTT
241	AGGTCAAGGT	GTAGCTTATG	GGATGGAGAG	AAATGGGCTA	CATTTTCTAC	TACAAGAACA
301	ACAATTATCC	AAACGAAAGC	CCCCATGAAA	CTAAGGGCTA	AAGGAGGATT	TAGCAGTAAA
361	TTAAGAACAG	AGAGCTTAAT	TGAACAAGGC	CATAAAGCAC	GC	
SumRhinoB12S						
1	GCTTAGCCCT	AAACCTAAAT	GATTTCCCCC	AACAAAATCA	TTCGCCAGAG	TACTACTAGC
61	AATAGCCTAA	AACTCAAAGG	ACTTGGCGGT	GCTTTATATC	CCCCTAGAGG	AGCCTGTTCC
121	ATAACCGATA	AACCCCGATA	AACCTTACCA	ACCCTTGCTA	ATTCAGCCTA	TATACCGCGA
181	TCTTCAGCAA	ACCGTAAAAA	AGGAACTAAA	GTAAGCACAA	GTATAAGACA	TAAAAACGTT
241	AGGTCAAGGT	GTAGCTTATG	GGATGGAGAG	AAATGGGCTA	CATTTTCTAC	TACAAGAACA
301	ACAATTATCC	AAACGAAAGC	CCCCATGAAA	CTAAGGGCTA	AAGGAGGATT	TAGCAGTAAA
361	TTAAGAACAG	AGAGCTTAAT	TGAACAAGGC	CATAAAGCAC	GC	
SumRhinoWM12S						
1	GCTTAGCCCT	AAACCTAAAT	GATTTCCCCC	AACAAAATCA	TTCGCCAGAG	TACTACTAGC
61	AATAGCCTAA	AACTCAAAGG	ACTTGGCGGT	GCTTTATATC	CCCCTAGAGG	AGCCTGTTCC
121	ATAACCGATA	AAGCCCGATA	AACCTTACCA	ACCCTTGCTA	ATTCAGCCTA	TATACCGCCA
181	TCTTCAGCAA	ACCCTAAAAA	AGGAACTAAA	GTAAGCACAA	GTATAAGACA	TAAAAACGTT
241	AGGTCAAGGT	GTAGCTTATG	GGATGGAGAG	AAATGGGCTA	CATTTTCTAC	TACAAGAACA
301	ACAATTATCC	AAACGAAAGC	CCCCATGAAA	CTAAGGGCTA	AAGGAGGATT	TAGCAGTAAA
361	TTAAGAACAG	AGAGCTTAAT	TGAACAAGGC	CATAAAGCAC	GC	
SumRhinoB16S						
60	CACCTCTAGC	ATACCCAGTA	TTAGAGGCAC	TGCCTGCCCA	GTGACATCTG	TTTCAACGGC
120	CGCGGTATCC	TAACCGTGCA	AAGGTAGCAT	AATCACTTGT	TCTCTAAATA	AGGACCTGTA
180	TGAATGGCCA	CACGAGGGTT	TTACTGTCTC	TTACCTTCAA	TCAGTGAAAT	TGACCTCCCC
240	GTGAAGAGGC	GGGGATAACG	CAACAAGACG	AGAAGACCCT	ATGGAGCTTC	AATTAACTAA
300	TTCACAAAAA	CAAAACCTTC	AACCTATATC	TAAGGAATAA	CAAAATTTCG	ATTGAATTAG
360	CAATTTCGGT	TGCGGTGACC	TCGGAGAACA	AAACAACCTC	CGAGTGATTA	AATTCTAGAC
420	TAACCAGTCA	AAAATAATAC	ATCACTTATT	GATCCAAATT	ATTGATCAAC	GGAACAAGTT
480	ACCCTAGGGA	TAACAGCGCA	ATCCTATTCT	AGAGTCCATA	TCGACAATAG	GGTTTACGAC
540	CTCGATGTTG	GATCAGGACA	TCCTAATGGT	GTAACCGCTA	TTAATGGTTC	GTTTGTTCAA
553	CGATTAAAGT	CCT				
SumRhinoS16S*						
60	CACCTCTAGC	ATACCCAGTA	TTAGAGGCAC	TGCCTGCCCA	GTGACATCTG	TTTCAACGGC
120	CGCGGTATCC	TAACCGTGCA	AAGGTAGCAT	AATCACTTGT	TCTCTAAATA	AGGACCTGTA
180	TGAATGGCCA	CACGAGGGTT	TTACTGTCTC	TTACCTTCAA	TCAGTGAAAT	TGACCTCCCC
240	GTGAAGAGGC	GGGGATAACG	CAACAAGACG	AGAAGACCCT	ATGGAGCTTC	AATTAACTAA
300	TTCACAAAAA	CAAAACCTTC	AACCTATATC	TAAGGAATAA	CAAAATTTCG	ATTGAATTAG
360	CAATTTCGGT	TGGGGTGACC	TCGGAGAACA	AAACAACCTC	CGAGTGATTA	AATTCTAGAC
420	TAACCAGTCA	AAAATAATAC	ATCACTTATT	GATCCAAATT	ATTGATCAAC	GGAACAAGTT
480	ACCCTAGGGA	TAACAGCGCA	ATCCTATTCT	AGAGTCCATA	TCGACAATAG	GGTTTACGAC
540	CTCGATGTTG	GATCAGGACA	TCCTAATGGT	GTAACCGCTA	TTAATGGTTC	GTTTGTTCAA
553	CGATTAAAGT	CCT				
SumRhinoS16S						
60	CACCTCTAGC	ATACCCAGTA	TTAGAGGCAC	TGCCTGCCCA	GTGACATCTG	TTTCAACGGC
120	CGCGGTATCC	TAACCGTGCA	AAGGTAGCAT	AATCACTTGT	TCTCTAAATA	AGGACCTGTA
180	TGAATGGCCA	CACGAGGGTT	TTACTGTCTC	TTACCTTCAA	TCAGTGAAAT	TGACCTCCCC
240	GTGAAGAGGC	GGGGATAACG	CAACAAGACG	AGAAGACCCT	ATGGAGCTTC	AATTAACTAA
300	TTCACAAAAA	CAAAACCTTC	AACCTATATC	TAAGGAATAA	CAAAATTTCG	ATTGAATTAG
360	CAATTTCGGT	TGCGGTGACC	TCGGAGAACA	AAACAACCTC	CGAGTGATTA	AATTCTAGAC
420	TAACCAGTCA	AAAATAATAC	ATCACTTATT	GATCCAAATT	ATTGATCAAC	GGAACAAGTT
480	ACCCTAGGGA	TAACAGCGCA	ATCCTATTCT	AGAGTCCATA	TCGACAATAG	GGTTTACGAC
540	CTCGATGTTG	GATCAGGACA	TCCTAATGGT	GTAACCGCTA	TTAATGGTTC	GTTTGTTCAA
553	CGATTAAAGT	CCT				
SumRhinoWM16S						
60	CACCTCTAGC	ATACCCAGTA	TTAGAGGCAC	TGCCTGCCCA	GTGACATCTG	TTTCAACGGC
120	CGCGGTATCC	TAACCGTGCA	AAGGTAGCAT	AATCACTTGT	TCTCTAAATA	AGGACCTGTA
180	TGAATGGCCA	CACGAGGGTT	TTACTGTCTC	TTACCTTCAA	TCAGTGAAAT	TGACCTCCCC
240	GTGAAGAGGC	GGGGATAACG	CAACAAGACG	AGAAGACCCT	ATGGAGCTTC	AATTAACTAA
300	TTCACAAAAA	CAAAACCTTC	AACCTATATC	TAAGGAATAA	CAAAATTTCG	ATTGAATTAG
360	CAATTTCGGT	TGGGGTGACC	TCGGAGAACA	AAACAACCTC	CGAGTGATTA	AATTCTAGAC
420	TAACCAGTCA	AAAATAATAC	ATCACTTATT	GATCCAAATT	ATTGATCAAC	GGAACAAGTT
480	ACCCTAGGGA	TAACAGCGCA	ATCCTATTCT	AGAGTCCATA	TCGACAATAG	GGTTTACGAC
540	CTCGATGTTG	GATCAGGACA	TCCTAATGGT	GTAACCGCTA	TTAATGGTTC	GTTTGTTCAA
553	CGATTAAAGT	CCT				

Four haplotypes were identified in 17 rhinoceros samples. Localities: S, S = Sumatra; WM = West Malaysia; B = Borneo.

TABLE 3. Sumatran rhino variable nucleotide sites*

Site number	SS	SS*	SW	SB
133 (12S)	C	C	G	C
179 (12S)	C	C	C	G
194 (12S)	C	C	C	G
313 (16S)	C	G	G	C

SS = Sumatran rhinos #22, 24, 27, 28, 33; SS = Sumatran rhino #6; SW = Sumatran rhinos #1, 7, 13, 15, 19, 20, 23; SB = Sumatran rhinos #17, 26, 31, 38.

and two haplotypes from the animals on Sumatra. Four sites were variable (Table 3). These sites were position #133, 179, and 194 in the 12S sequence and position #313 in the 16S fragment. In total, the Bornean haplotype differed by two positions from Sumatran and three positions from West Malaysian. West Malaysia and Sumatra vary by one position for one of the Sumatran haplotypes and by two positions for the other Sumatran haplotype.

None of the positions, when considered individually, fit the definition of character as defined by Davis and Nixon [1992] (Table 3). Rather, they would be considered traits. If the suite of substitutions is considered an attribute, then one character supports the separation of the three defined populations (with a polymorphic Sumatra).

These few variable sites show a greater similarity between West Malaysia and Sumatra than either of those populations compared to Borneo. Position #179 and 194 supports Groves's [1967] subspecies designation placing the Malayan and Sumatran populations together as *D.s. sumatrensis* with the Borneo population as *D.s. harrissoni.*

DISCUSSION

The results of the population aggregation analysis (PAA) of Sumatran rhinos for determining conservation units were equivocal. Single sites were homoplastic and thus not characters by a PAA definition. The use of an entire haplotype as a single character is complicated by the fact that the population on Sumatra is represented by two haplotypes. If we consider these two haplotypes as character states, then we have a single character support for three phylogenetic species at the minimum level of distinction.

It is interesting but not surprising that the populations on West Malaysia and Sumatra appear slightly more similar than either does to Borneo. The isolation of Borneo by the submersion of the Sunda Shelf probably occurred a little earlier than the isolation of Sumatra from the mainland [Whitten et al., 1987]. In general, there is a trend of increasing morphological differences in birds and mammals as one proceeds from mainland Southeast Asia out along the Indonesian archipelago until the abrupt change that occurs in Sulawesi [Whitten et al., 1987]. A number of authors have described this as originally reflecting a cline through the areas that are part of the Sunda Shelf that were last connected about 12,000 years ago.

The question of determining conservation units is complicated in this particular case [Amato et al., 1993; Amato and Ryder, 1993; Amato and Wharton, 1993; and Wharton and Amato, 1993]. The populations are currently isolated on the mainland

(West Malaysia) and on two islands (Borneo and Sumatra). This temporal and spatial separation is sufficient reason to refer to these populations as separate for taxonomic purposes. However, with the goal of preserving the evolutionary novelty that is represented in the *Dicerorhine* lineage, can we consider the three populations as part of the same conservation unit? Applying the PAA assessment of phylogenetic species does not argue against diagnosing them as a single conservation unit unless we consider the Sumatran haplotypes as character states. If we consider the haplotypes as a single character supporting three phylogenetic species, it clearly is the weakest support possible from this data set. These same gene regions (12S and 16S) have shown fixed sequence differences between closely related bovid species [Gatesy et al., 1992] and subspecies of crocodilians [Amato and Gatesy, 1994]. Expanding the research to more variable regions is problematic due to the available number of samples. Since the three existing populations are greatly reduced in number, the chances of identifying highly variable characters that unite them simply because the intermediates are missing is likely. Also, traits that unite small, fragmented populations can reflect inbreeding or the localized presence of a rare mutation in related individuals.

Groves's [1967, 1993] subspecies designations are based on only eight morphological characters (all measurements as opposed to presence or absence) using a smaller sample size than this study. His West Malaysian and Sumatran measurements overlap extensively. Only Borneo is less similar. The results reported in this paper are not in serious conflict with the results from Groves's [1967] morphological data.

The only other large mammal that has a similar distribution, and that has been assessed on status as subspecies/conservation unit, is the orangutan (*Pongo pygmaeus*). Orangutans are found on both Sumatra and Borneo (and prehistoric remains have been found on the mainland) and may be assumed to have been isolated for the same length of time. Three studies [Caccone and Powell, 1989; Janczewski et al., 1991; Ryder and Chemnick, 1993] support the division of the two orangutan populations into minimally distinct species. This apparent conflict with the Sumatran rhino results may reflect such factors as generation time, the orangutan's obligate arboreal life style, and differences in dispersal abilities, among others. It is worth noting that the two orangutan populations interbreed readily and successfully in captivity, with no signs of reduced fitness after several generations.

It is also worth noting that rhinoceros are chromosomally very conservative [Houck et al., 1994]. Indian, Sumatran, and white rhinos all have a karyotype of $2n = 82$ even though they last shared a common ancestor more than 15 million years ago. This chromosomal conservation reduces concerns about cytogenetic incompatibility.

There is no strong evidence supporting more than one conservation unit for Sumatran rhinos. Chromosomal conservation and degree of sequence divergence make outbreeding depression [Templeton, 1986] an unlikely outcome if individuals, or their gametes, are translocated as part of a conservation management plan. While this research, like all scientific research, is falsifiable by the addition of further data, it is unwise to be paralyzed into inaction while waiting for more studies. The question of when enough studies have been conducted to "prove" that there is only one conservation unit becomes a question of trying to prove rather than reject the null hypothesis. This is an epistomological problem rather than a scientific problem and should not prevent us from developing a conservation management plan to preserve this unique taxon.

The importance of this study is in providing support for flexibility in our management options. There is no evidence from this study, or any other study, to suggest that there would be biological problems resulting from the interbreeding of Sumatran rhinos from different parts of their range. That is not to say that other molecular markers might not identify subdivision below the species level. However, while most local populations reflect varying degrees of subdivision (and certainly the geographically isolated populations are not currently exchanging genes), this does not mean that we should treat each local population of every species as our unit of conservation. While there is as yet no immediate urgency to exchange animals among the three regional in situ populations, the current captive population would clearly benefit from exchanges in order to address uneven sex ratios. It is our recommendation that proposals to move animals between regional plans that would likely increase reproduction be acted upon immediately.

CONCLUSIONS

1. Evidence for significant evolutionary differences between geographically separated populations of Sumatran rhinos based on mitochondrial DNA sequence divergence and morphological characters is lacking.

2. The threat of extinction of the evolutionarily distinct *Dicerorhine* lineage is high.

3. Animals should be moved between regional ex situ plans and into protected reserves in order to maximize opportunites for reproduction and maintain demographically and genetically healthy populations, regardless of historical subspecies designations.

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