IUCN/SSC
ASIAN RHINO SPECIALIST GROUP
(AsRSG)
MEETING

BRIEFING BOOK

JALDAPARA SANCTUARY
WEST BENGAL, INDIA
6-10 DECEMBER 1993

SECTION 20
SUBSPECIES ISSUES

A PROPOSAL FOR A PROCESS AND WORKSHOP ON DEFINING CONSERVATION UNITS FOR RHINOCEROS - APPLYING SCIENCE TO CONSERVATION POLICY

George Amato and Tom Foose
Revised - 1 December 1993

A process and workshop to assemble scientists researching "subspecies" questions relevant to conservation and/or conducting research on rhinoceros genetics/systematics is proposed for March 1994. The workshop would require two days and be conducted at the American Museum of Natural History in New York or the White Oak Conservation Center, Yulee, Florida.

All five species of rhinoceros are greatly threatened. Since they are high profile species, there has been a great deal of effort and money expended to help preserve these species in situ and ex situ. One of the major complicating factors in this effort has been the confusion of how many conservation units are we dealing with -- the dilemma of subspecies. Subspecies issues are becoming particularly critical for conservation of both black and Sumatran rhino, both in captivity and in the wild.

The IUCN SSC African and Asian Rhino Specialist Groups have been recognized as the authority determining the policy that has guided both wild and captive programs. To date, this policy has been to recognize all the maximal number of described or presumed subspecies as the conservation units to be conserve separately. The subspecies distinctions have been based on morphological traits and geographical distribution.

It has been recognized that genetic information would provide very useful addition information to assess the validity of these units. Hence, much genetic analysis by a number of researchers using a variety of techniques has been in progress for both Sumatran and black. Some preliminary results have been reported but have been inconclusive in part because of inadequate samples. Consequently, subspecies policy has not changed although there have been suggestions it should. More complete analyses with better sample sizes are now near completion for both species.

In anticipation of this data, several persons (Amato, Foose, Willis) have been discussing a process that may help resolve continuing issues. It is proposed to compile and collate the results of all analyses, published and unpublished. It is then proposed to convene a small workshop of geneticists and systematists that have been concerned with rhino subspecies issues to assess this data and more importantly to propose a decision process whereby all relevant information (genetic, morphological, and geographical) can be used to recommend what the valid conservation units are.

Beyond the genetic data, consideration of possible ecological differences is also important. So far the black rhino subspecies issue has revolved almost entirely around conventional morphometric studies and biochemical work in various laboratories. Africa's black rhinos occupy a range of habitats from high-altitude forest to desert. Whatever the measurable genetics indicate, it is unwise to assume that rhinos have not developed physiological and behavioral adaptations in these different habitats. An ecological perspective must be included in the process of defining the conservation units.

The results of this process would then be communicated to the various Regional Rhino Taxon Advisory Groups and Breeding Programs and particularly to the IUCN SSC Rhino Specialist Groups, which presumably will continue to be recognized as the governing authority on this matter.

This workshop would allow review of the current data, while at the same time provide a forum for clearly articulating our goals and methods for dealing with subspecies issues. These include:

1. What is a subspecies/E.S.U./conservation unit (i.e. any population diagnosably distinct, or if minimally distinct should we characterize the levels of genetic differentiation?)?
2. Should our strategy for determining conservation units in captive programs reflect exactly, largely, or not at all our strategy for in situ programs. Additionally, arguments for what sorts of captive populations are most wanted – or likely to be successful for reintroductions will need to be considered.
3. How do we conserve the most evolutionary novelty? Is this a primary goal?

Prior to the workshop, invited participants will be provided with a compilation of relevant papers as well as unpublished data which has been solicited from the various researchers. The format of the workshop will be an iterative series of plenary discussions and working group sessions.

The objective will be to develop a consensus on subspecies issues for rhinoceros and to produce a set of recommendations based on biological considerations that can then be submitted to policy development authorities: i.e., the IUCN African and Asian Rhino Specialist Groups, both of which convene in later 1993, and the Rhino Taxon Advisory Groups of the Regional Captive Conservation Programs worldwide (e.g., AAZPA, EEP, ASMP, etc.) In the process, a model for moving forward on defining conservation units for other species may emerge.

Intended invitees include:

George Amato
George Barrowclough
Evan Blumer
Joel Cracraft
Raoul du Toit
Tom Foose
Eric Harley
Bob Lacy
Lynn Maguire
Juan Carlos Morales
Norman Owen-Smith
Ollie Ryder
Nico Van Strien
Dan Wharton
Bob Wayne
Kevin Willis

Workshop costs are estimated at $ 10,000 for travel expenses of "out-of-town" participants and for publication costs.

A SYSTEMATIC APPROACH TO IDENTIFYING UNITS OF CONSERVATION: EXAMPLES OF PROGRESS AND PROBLEMS

George D. Amato and Dan Wharton
NYZS/The Wildlife Conservation Society

SUMMARY

A number of authors have recently suggested that the best approach for identifying units of conservation is to follow a systematics model of character analysis (Amato, 1991; Cracraft, 1991; Vogler and DeSalle, in press). This approach necessitates the use of an operational, typological evolutionary species concept. The use of the phylogenetic species concept has the utility and philosophical logic appropriate for this task. Additionally, there is a large body of literature that uses this framework, along with a parsimony character analysis to identify patterns of phylogeny (Cracraft, 1983; Nelson and Platnick, 1981; Nixon and Wheeler, 1990).

While we strongly advocate this approach, we recognize that it has both strengths and weaknesses depending on the specific research project. Presented here are a comparison of two such conservation unit research projects that have been conducted at NYZS/The Wildlife Conservation Society, which demonstrate both the progress in techniques and the difficulties with which we are still confronted.

IMPORTANT TECHNOLOGICAL PROGRESS

If phylogenetic species are to be our units of conservation (see Wharton and Amato, this volume), it is necessary to identify characters for analysis. While all types of characters (morphological, behavioral, karyotypic, and genetic) are useful and important, the recent advances in molecular biology have provided powerful new tools for character analysis. Polymerase Chain Reaction (PCR) technology has been especially important for allowing researchers to easily obtain DNA sequence data--providing enormous numbers of genetic characters useful for a systematics approach to identifying conservation units. Along with this improved ability to generate large numbers of characters has been an explosion of ideas and algorithms for analyzing molecular character data for phylogenetic study (Swofford, 1990; Felsenstein, 1990).

Whereas identifying a phylogenetic species rests only on demonstrating that a population has diagnosable characters (which are shared by all members but are not found in other groups), the use of phylogeny reconstruction may be important in identifying useful regions for characterization (Amato et al., 1993).

Another important development with PCR has been the use of PCR to obtain target DNA sequences from nontraditional biological samples. Before PCR, biochemical techniques required careful preparation of large quantities of fresh blood or organ tissues (for vertebrates). This proved especially problematic for conservation research because samples were often needed from animals that were handled infrequently, existed only in small, isolated populations, and might only be handled by field researchers who had difficulty in obtaining and preserving biological samples. PCR advances allowed for the use of such samples as hair, small skin biopsies, shed feathers, dried blood, museum specimens, and others (Amato et al., 1993; Walsh et al., 1991; Garza and Woodruff, 1992). Now with the collection of materials made easier, as well as the generation of large numbers of molecular genetic characters, we are better prepared than ever to tackle questions concerning units of conservation.

SOME EXAMPLES OF CONSERVATION UNIT RESEARCH EMPLOYING A PHYLOGENETIC SPECIES IN A SYSTEMATICS FRAMEWORK

Summaries from two research projects are presented: Sumatran rhinoceros (Dicerorhinus sumatrensis) and the common caiman (Caiman crocodilus). Sequence data has been deposited in GENBANK. Detailed descriptions of methods have been published and are in preparation (see ref.).

Sumatran Rhinoceros:

In our study of Sumatran rhinoceros we analyzed 974 bases of mitochondrial sequence for seven animals from peninsular Malaysia, six animals from Sumatra, and four animals from Borneo. This data represents portions of the two small ribosomal subunits (12S and 16S), and a protein coding gene (cytochrome b). A total of seven polymorphic bases was observed. Two synapomorphies were shared by the four Bornean samples. Only one synapomorphy unites the Sumatran samples but the observed haplotypes (two) were more similar to each other than to the other two populations. Similarly, the haplotypes on peninsular Malaysia were more similar to each other than to the Bornean and Sumatran samples.

All available samples have been included in this study. More than half of the samples were available only as recently collected hair or dried, shed skin. The only blood samples collected were preserved in a buffered solution that enabled them to be stored and shipped at room temperature (without special handling or storage). Without the previously mentioned improvements in technology, this research would have been impossible. Additionally, direct sequencing has provided a large number of characters for analysis. However, in spite of the increased opportunity for sampling by the use of hair samples, the sample sizes are still small.

Davis and Nixon (1992) suggest a methodology for assessing which attributes are phylogenetic characters and which are traits. This methodology (population aggregation analysis) involves successive searches for fixed differences between aggregations of local isolated populations. Characters are attributes that exist as fixed, unique differences in an extended genealogical population. Traits are attributes that are not unique to a population, or may be polymorphic. Davis and Nixon (1992) correctly point out that cladistic methodology allows for creating a hierarchy out of the terminal units by scoring attributes, but that only characters, not traits, can be used for determining a hierarchy that has phylogenetic information. Characters are what identify phylogenetic species. Furthermore, they observe that small sample sizes can affect our ability to discriminate between a character and a trait such as with the Sumatran rhinoceros, which persist in highly fragmented populations. All of the individuals in a population of rhinos (such as the thirty or less found on Borneo) may be closely related and share attributes that reflect those familial relationships. These attributes would not be informative about the populations evolutionary distinctiveness or history.

Since we have stated that species should be our unit of conservation, we must assess our data to see if we have refuted the species status of Dicerorhinus sumatrensis by supporting the occurrence of two or three species of closely related rhinos. We would argue that the number and distribution of attributes scored does not offer compelling evidence of the presence of three phylogenetic species. Clearly we are hampered in our assessment of which attributes are characters due to the available sample size. However, it would be misleading, not simply subjective, to ignore this limitation. Furthermore, the problems of small sample size cannot be overcome by adding additional sequence from the available samples. The problem is the effect of missing individuals, and their impact in identifying patterns of attributes.

Caiman crocodilus:

In this study, more than 70 samples of Caiman crocodilus, collected from wild populations across the species' entire range were analyzed. These samples were a subset of samples collected by Peter Brazaitis and colleagues as part of a long term field study of the distribution of crocodilians in South America (Brazaitis et al., 1990; 1992). The project will eventually include an analysis of approximately 400 samples of known locality.

Initially, thirty samples were completely sequenced for 12S and 16S ribosomal mitochondrial genes. Six of 650 bases were polymorphic and yielded three haplotypes. Each haplotype corresponded to a morphologically diagnosed subspecies by Brazaitis (1992). Base specific primers were constructed to screen for polymorphic base identity by PCR amplification (Amato and Gatesy, in press). Positive and negative controls were used for all samples, with any problematic samples being completely sequenced. The originally identified diagnosable sites were found in the additional 50 samples screened. At this time we are adding cytochrome b sequence as well as additional samples.

The use of PCR based techniques allowed us to identify a large number of characters for this caiman study. In addition, since many of these valuable samples were only available as dried blood, the use of PCR with chelex isolated DNA (Walsh et al.,1991) allowed us to greatly expand our sample size. Also, the ability to screen large numbers of samples for presence of specific bases provided a more rapid and less costly approach to assessing whether a polymorphic site was a trait or character. The number of samples and the distribution of characters suggests a powerful argument for identifying three phylogenetic species and units of conservation. This data also has potential important forensics applications for a species group that is involved in commercial trade (Brazaitis pers. comm.).

DISCUSSION

Now that the approach of using phylogenetic species as a guide for identifying units of conservation has been proposed (Vogler and DeSalle, in press; Barrowclough and Flesness, in press; Amato, 1991; Wharton and Amato, this volume), we need to clearly identify any weaknesses inherent in this approach. We attempted to use two studies from our research to contrast more and less successful uses of this strategy.

Cracraft (1991; and pers. comm.) has argued that the only way to objectively identify units of conservation is to use a phylogenetic species/lower level systematics approach. Discriminating between the significance of diagnosable characters would be subjective. While we feel strongly that this approach should provide the framework for identifying units of conservation we are concerned by some of the limitations. As pointed out in the rhino study, we are concerned about discriminating patterns of attributes in a species that now only exists in highly fragmented, small populations in comparison to its historic distribution. The black rhinoceros population was estimated to be over 60,000 in 1970 (already greatly reduced from 1900), and now is less than 2,500. To survey scattered populations of animals that may number as few as ten animals for diagnosable characters may yield patterns that do not reflect evolutionary events. Character data may have to be discussed in the context of additional data sets that could provide important insights into reconstructing original patterns. This additional data may be historical accounts of the original distribution, information about the relative rates of evolution for particular regions or sites based on higher level phylogenies, or perhaps molecular characters from museum specimens representing areas where the animals have been locally extirpated.

While this may sound too subjective to some authors, we would argue that decisions on units of conservation are ultimately subjective. That does not prevent us from employing a rigorous framework from which to improve what admittedly will be a subjective decision. Davis and Nixon (1992) attempt to overcome some of the problems with identifying phylogenetic species with their assessment of attributes as traits or characters. However, Davis and Nixon (1993) acknowledge the problem of sample size in this analysis; and more importantly, fail to consider the subjective nature of operationally identifying what constitutes a population. If a rigorous objective phylogenetic species definition rests on a subjective assessment of a population's boundary, is the accusation of subjectivity for identifying units of conservation a fair criticism?

The study of Caiman crocodilus, however, demonstrates a more successful use of this approach. By having a large number of samples from a group still present in much of its original range, we overcame many of these problems. With a wide distribution of diagnosable molecular characters that are congruent with morphological characters and biogeographical data, we have much greater confidence in our results.

While the approach outlined rests on a sound foundation of logic and rigorous science, we have identified that it suffers some limitations when applied to very closely related taxa, small data sets, and operationally defined populations--precisely the situations we most frequently confront in conservation. However, it is still a less problematic approach than using overall similarity or genetic distances which have been shown to be largely uninformative for identifying taxonomic rank or units of conservation (Vogler and DeSalle, in press; Avise and Acquadro, 1982).

Only continued research using this approach, followed by decision-making and action by managers will ultimately demonstrate its usefulness. Systematics provides us with an important framework to aid in identifying our conservation units where as population genetics provides us with useful models for managing these units. It is the managers in zoological parks, governments, and international conservation organizations that must use these results to implement the important management actions.

Acknowledgements

We thank P. Brazaitis, J. Gatesy, and R. DeSalle for contributions to the research reported. R. DeSalle and J. Gatesy also contributed through countless discussions. J. Gleason and J. Gibbs provided helpful comments on the manuscript.

Cracraft, J.A. 1989. Speciation and ontology: the empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. Pp. 28-59 in: D. Otte and J. A. Endler (eds.) Speciation and its Consequences. Sinauer Associates, Sunderland, MA.

Cracraft, J. 1991. Systematics, species concepts, and conservation biology. Abstract. Society for Conservation Biology, 5th Annual meeting

Davis, J.I., and K. C. Nixon. 1992. Populations, Genetic variation, and the delimitation of phylogenetic species. Systematic Biology vol.41 4:421-435.

Felsenstein, J. 1990. PHYLIP: Phylogeny Inference Package, version 3.3, University of Washington, Seattle, WA.

Garza, J.C., and D.S. Woodruff. 1992. A phylogenetic study of the gibbons (Hylobates) using DNA obtained noninvasively from hair. Molecular Phylogenetics and Evolution vol. 1 3:202-210.

Madison, W.P., M.J. Donoghue, and D.R. Madison. 1984. Outgroup analysis and parsimony. Systematic Zoology 33:83-103.

Nelson, G. J., and N.I. Platnick. 1981. Systematics and biogeography: cladistics and vicariance. Columbia University Press, New York.

Nixon, K.C., and Q. D. Wheeler. 1990. An amplification of the phylogenetic species concept. Cladistics 6:212-223.

Kocher, T.C., W.K. Thomas, A. Meyer, S.V. Edwards, S. Paabo, F.X. Villablanca and A.C. Wilson. 1989. Dynamics of mitochondrial evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86:377-382.

Ryder, O.A. 1986. Species conservation and systematics: the dilemma of subspecies. Trends in Ecology and Evolution. Vol. 1 pg. 9-10.

References

Amato, George D. 1991. Species hybridization and protection of endangered animals. Science 253 (5017):250.

Amato, George D., Mary V. Ashley, and John Gatesy. 1993. Molecular evolution in living species of rhinoceros:Implications for conservation. Proceedings of the International Conference on Rhinoceros Conservation and Biology. O.A. Ryder editor. Zoological Society of San Diego.

Avise, J.C., and C.F. Aquadro. 1982. A comparative summary of genetic distances in the vertebrates. Evolutionary Biology 15:151-185.

Barrowclough, G.F. and N.R. Flesness. 1993. Species, subspecies, and races:The problem of units of management in conservation. In M. Allen and H. Harris (eds.) Wild Mammals in Captivity. University of Chicago Press. In press

Brazaitis, P., G. Rebelo, and C. Yamashita. 1990. A summary report of the CITES central South American caiman study: Phase I: Brazil. In Crocodiles, Proceedings of the 9th Working Meeting of the Crocodile Specialist Group, IUCN. Gland< Switzerland. 1:100-115.

Brazaitis, P., G. Robelo, and C. Yamashita. 1992. Report of the WWF/Traffic USA Survey of Brazilian Amazonia Crocodilians: Survey period July 1988 to January 1992.

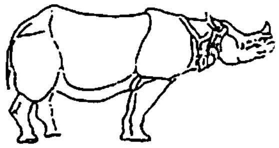
Caccone, A., G.D. Amato and J.R. Powell. 1987. Intraspecific DNA divergence in Drosophila: a case study in parthenogenetic D. mercatorum. Mol. Biol. Evol. 4:343-350.

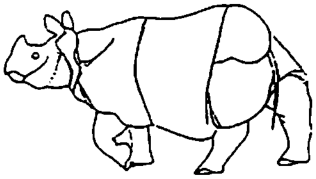
Cracraft, J.A. 1983. Species concept and speciation analysis. Current Ornithology 1:159-187.

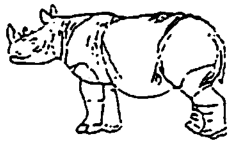
Swofford, D.L. 1990. PAUP: Phylogenetic Analysis using Parsimony, version 3.0.Computer program distributed by Illinois Natural History Survey. Champaign IL.

Walsh, P.S., D.A. Metzger and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10(4):506-513.

IUCN SSC ASIAN RHINO SPECIALIST GROUP







Chairman:
Mohd Khan bin Momin Khan
Forestry Department, ITTO Unit
Fifth Floor, Wisma Sumber Alam
Putra Jaya, Jalan Stadium 93660
Kuching, Sarawak, MALAYSIA
Telephone: 60-82-445105
Fax: 60-82-445639

Program Officer:
Thomas J. Foose, Ph.D.
International Rhino Foundation
The Wilds
85 E. Gay St., Suite 603
Columbus, OH 43215, USA
Telephone: 1-614-228-0402
Fax: 1-614-228-7210

10 September 1993

TO: Wolfgang Frey

FROM Tom Foose

SUBJECT: YOUR LETTER OF 24 AUGUST 1993 TO MOHD KHAN

Thank you for your letter of 24 August to Mohd Khan concerning subspecies of *Rhinoceros unicornis*. Mohd Khan has requested that I respond to your letter. I am currently assisting Mohd Khan as program officer for the Group.

The general issue of rhinoceros subspecies is a matter of very active interest and investigation at the moment. Greatest attention has been directed to *Dicerorhinus sumatrensis* and *Diceros bicornis* because these are the two species where the subspecific distinction in relation to the conservation crisis for the species has seemed the most urgent. Moreover, there has been a tendency to emphasize genetic rather than morphological information in recent considerations.

To my knowledge the genetic work that has been done on the Great One-Horned Asian Rhinoceros has not demonstrated any significant differences. However, I have referred you letter to Dr. George Amato of the New York Wildlife Conservation (Zoological) Society and Yale, who has emerged as one of the leading genetic researchers on rhino subspecies problems for further comment.

You probably are aware of the paper by Colin Groves that does detect morphological differences between the Assam and Nepal specimens and recommends subspecific distinction. The paper is in the Proceedings of the International Conference on Rhinoceros Biology and Conservation that was conducted in San Diego in 1991. For convenience a copy is attached.

Dr. Amato and I are attempting to organize a more rigorous process and workshop to consider rhinoceros subspecies issues. We are hopeful this may occur before the next meeting of the Asian Rhino Specialist Group which will be in India 6-10 December 1993. I'm sure the subspecies issue will be considered then, especially in relation to the Dudhwa Reintroduction Project. We'll keep you informed.

In the meantime, can you provide any further information about the "reliable reports" of Sumatran Rhino in western Sichaun. Thanks very much.

cc: Mohd Khan, G. Amato.

IUCN
The World Conservation Union

NYZS The WILDLIFE CONSERVATION SOCIETY
THE INTERNATIONAL WILDLIFE CONSERVATION PARK/BRONX ZOO • BRONX, NY 10460-1099 USA • (212) 220-5100

April 27, 1993

Dr. Zainal Zahari Zainuddin
Zoo Melaka

Dear Dr. Zainuddin:

I have completed an analysis of the mitochondrial DNA sequence variation in the Sumatran rhino samples that have been provided to me. The animals included in the study are:

Panjang
Mas Merah
Jeram
Rima
Shah
Minah
Seputih

Rapunzel
Bagus
Dalu
Rokan
(two other Sumatran animals that died in U.S.A.)

Takala
Tanegang
Sidom
Lun Parai

They appear minimally distinct--that is the individuals sampled have mitochondrial genotypes more similar to others from the same population than to individuals of other populations. The degree of divergence, I believe, is insufficient to warrant a separate captive breeding program for each population. I would maintain the captive breeding group as a single population, concentrating on husbandry techniques and matching compatible pairs. If there are subtle local adaptations, they will probably not be meaningful in a captive environment. We will retain greater genetic diversity if we can manage to have founder representation of all individuals currently in the captive population. If it becomes practical to reintroduce animals, they will have a greater degree of genetic diversity from this management strategy and will be amenable to the sorting that accompanies natural selection in their new environment.

Tom Foose and I are trying to convene a meeting of scientists specializing in subspecies questions to meet in New York this summer. We will review and discuss this data as well as data on other species of rhinos. The results of this meeting will be presented to the rhino specialist groups, AAZPA, etc. In the meantime, I am continuing to gather data from any new samples (including museum specimens) that become available. When I write the paper, I will send you a draft for comments. Of course I would like you to be a coauthor on the Sumatran rhino work. It would have been impossible without your help.

Leslie Johnston informed us that you could use some blood transfusion needles for sampling. I have ordered some, and I will send them along when they arrive. Of course, any additional samples that become available would be greatly appreciated.

Thanks again for your help.

Best regards,

George Amato

THE SUBSPECIES DILEMMA: AN OVERVIEW AND RECOMMENDATION

Dan Wharton, Curator of Animal Management Services
George Amato, Conservation Geneticist
NYZS/The Wildlife Conservation Society
185th Street and Southern Blvd.
Bronx, NY 10460

The so-called "subspecies dilemma" (Ryder, 1986; Wharton, 1985) is the result of interplay among several phenomena: (1) the lack of urgency in traditional systematics which is, by definition, a descriptive science not oriented to conservation nor to crisis; (2) basic philosophical disagreements on the definition of species; (3) the frequent, unresolvable ambiguity of "evolutionary significance" among subspecies historically due to the limited number of morphological characters used by systematists for analyses; and (4) the perception of a single rule of subspecies management regardless of circumstances. Added to the dilemma is the fact that the "unit of conservation" (population vs. subspecies vs. species) has not been clearly articulated. The "evolutionarily significant unit" or ESU (Wharton, 1985; Ryder, 1986; Woodruff, 1989; O'Brien and Mayr, 1991; Amato, 1991) proposed in 1985 is helpful in stating that evolutionary parameters should define the conservation unit, but leaves open the question of "significance" (Vogler and DeSalle, in press).

TRADITIONAL SYSTEMATICS

Traditional systematics is central to the dilemma. We, as conservationists in the latter part of the 20th century, must react to an avalanche of wildlife crises all around us and with limited resources. While systematists may rightly be content to split or lump subspecies on paper as the data accumulates over time, conservationists must consider the literal actions of splitting and lumping from an animal management point of view. Errors in either action are costly: unnecessary splitting consumes limited resources while erroneous lumping or merging of distinct taxa is antithetical to conservation's role in preserving biodiversity (see Ryder et al., 1987; Shaw et al., unpubl).

Zoo biologists have been among the first to call attention to the subspecies dilemma since the captive breeding setting confronts the issue repeatedly with founder stock often coming from different parts of a species range and with different trinomials. As zoological garden efforts in the breeding of endangered species applied the science of population biology in "species survival plans," a major impasse in developing some breeding programs appeared where taxa could not be clearly defined. In 1985, several zoo biologists and systematists met at the Philadelphia Zoological Garden to discuss the problems and coined the term "evolutionarily significant unit" to distinguish populations that represented significant progress toward speciation versus those that did not (Wharton, 1985). However, the process by which this distinction was to be made was not clear cut and the recommendations of the meeting were essentially summarized as (1) the need to collect more data where ambiguity existed and (2) to remain conservative on subspecies management by maintaining subspecies separation where in doubt. Since that time, new techniques and methodologies have come about as have new perspectives on the process of evolution. These new developments in just eight short years offer a great deal to the subspecies dilemma although they have not substituted the need for formulation of clear policy on subspecies management.

THE SPECIES CONCEPT

Alfried Vogler and Rob DeSalle (in press) have offered excellent discussion on the problem of differing species concepts and their impact on conservation's ability to define the ESU or evolutionarily significant unit. There are several concepts which might be considered in this light, but Vogler and DeSalle argue that two are most prominent, with the most recent showing the greatest potential aiding our definition of the ESU: the Biological Species Concept (BSC, Mayr, 1942; 1963; 1969) and the Phylogenetic Species Concept (PSC, Nelson and Platnick, 1981; Cracraft, 1983; 1989). The older, traditional BSC under which most of us were trained defines species as "groups of actually or potentially interbreeding populations that are reproductively isolated from other such groups " (Mayr, 1969). The problem with this concept lies in its subjective application: (1) Since reproductive isolation cannot normally be observed, it must be inferred from morphological clues and (2) it assumes that morphological or genetic differentiation among populations is in direct proportion to the level of reproductive isolation, which they are not. Since the BSC cannot be applied objectively and because reproductive compatibility can occur in the presence significant degrees of differentiation, the BSC fails to provide the stable, conceptual framework with which to identify conservation's ESU.

On the other hand, the PSC is based on the simpler fact that living organisms are the sum of their genetic endowment with heritable characters that can guide taxonomic placement. Under this concept, an ESU is a population that is the evolutionary result of lineage separation, objectively diagnosable through a range of observable characters, phenotypic and/or molecular. The characters can range from changes in DNA to skull morphology to behavior. While critics of the concept see the selection of characters as somewhat arbitrary, the important element in employing the PSC is in selecting characters which clearly have a genetic basis.

CHARACTERS

At the time of the Philadelphia "subspecies dilemma" meeting in 1985, there was a sense that the ESU would be nearly impossible to identify in many cases. Upon careful analysis, it was expected that clinal variations would reveal themselves as would overlooked hemi- or full species. The large, gray area in-between represented by non-contiguous populations of easily recognized eco- or geo-types would continue to offer problems to conservation biologists. The primary difficulty was in the limited number of informative characters that one can assemble from an animal's morphology. In the 1970's, starch-gel electrophoresis (a biochemical technique that assesses protein variation) provided data for calculating genetic distances based on frequencies of alleles. However, this data is frequently uninformative for phylogenetic analyses and is generally poor character data. While this data may be useful for managing populations, it is less informative than current molecular data for a PSC approach to defining conservation units.

Molecular genetics and molecular systematics offer a major step forward in addressing the subspecies dilemma. By examining the genome of a random sampling of animals from different populations, one can actually address the PSC by adding additional characters which clearly have a genetic basis. Via many techniques, the number of characters available for inclusion in a data set is theoretically limited only by the size of the genome itself which in most vertebrates is 10^5 or 100,000 genes. Depending upon resources, it is now possible to pursue the question of inter-populational relatedness to a much higher degree of satisfaction.

The single most perplexing issue remains in the definition of "significant" in our ESU. As implied from the very first, significance is a relative term. "Diagnosably distinct" is often used to suggest ESU status; however, distinctive populations below the species level are not necessarily a prediction of separate evolutionary destinies since reproductive isolation is usually a matter of physical barriers that may or may not persist It is probably fair to suggest that the ultimate ESU is, of course, a full species since evolutionary destiny, as separate from other members of the genus, is fairly clear. The whole question of significance becomes moot if we accept that "subspecies" is in fact a descriptive term and not prescriptive. The responsibilities of the conservation biologist are in (1) settling the binomial vs. trinomial questions where they exist and (2) promoting management plans that primarily address species conservation using populations, including diagnosably distinct subspecies, as a means to that end.

SUBSPECIES MANAGEMENT

As much as is reasonably possible, species conservation strategies should recognize the expediency of preserving the binomial with the full spectrum of extant genetic variation. However, the methods may vary dramatically depending upon the degree of crisis and the opportunities available. Where habitat disturbance is largely controllable, the strategy of choice is *In situ* preservation of multiple populations across the species' entire natural range. At the other end of the spectrum is the collection of sub-viable remnants of single or multiple populations for rescue *ex situ* as a panmictic captive population. In both cases, the binomial remains the focus while the trinomials/populations are elements of very different strategies. Objective concerns regarding subtle, co-adapted gene complexes (Templeton, 1986; Templeton et al., 1986) remain relevant only as long as habitat remains relatively undisturbed and "adaptation" continues to be operational. Fragmented habitats, isolated reserves, and captivity all imply new rules of adaptation and, concomitantly, new rules of population management (Conway, 1989a; 1989b).

For instance, the white-tailed deer (*Odocoileus virginianus*) represents a common species with many, large populations/subspecies over a broad geographical area. In this case, management can default to stabilization of existing populations and a very rough approximation of historical levels of gene flow among populations (although some controversial mixing of populations continues to occur as a demographic management scheme; Wemmer, in press). Detailed analysis is not required to employ this strategy since it is largely an attempt to maintain a natural population dynamic. Although the black-footed ferret (*Mustela nigripes* represents a totally opposite case in endangerment status (population reduced to 17 animals prior to successful captive propagation), the management response is likewise uncomplicated by the subspecies issue (Seal et al., 1989). The difficult cases resemble that of the black rhino (Ashley et al., 1990; Amato et al., 1993; Harley and O'Ryan, 1993), reduced to approximately 2,500 animals representing five populations in Africa. Detailed molecular analysis clearly indicates that black rhino populations are not significantly differentiated despite some quantifiable morphological and ecological differences. Management plans currently follow the white-tailed deer pattern *in situ* and the *ex situ* response has been to keep the "subspecies" separate.

The conservative approach advocated by Seal et al. (1993) suggests that "splitting" rather than "lumping" maximizes future options. Taxa can always be merged (or lumped) later if further information invalidates the distinction or if dwindling numbers impose the decision. This approach, however, does not provide any specific rules of action once invalidation takes place. In the case of the black rhino, the course of action would be superficially the same although the invalidation of subspecies offers new direction in program planning. The decision to translocate specimens from a successful subpopulation to a faltering one need only be considered in terms of risk of losing all genetic representation in the faltering population without the outcross. Sacrifice (or potential sacrifice) of "evolutionary integrity" is no longer a consideration as it must remain in the absence of investigation. *Ex situ* management strategies are able to consider specimens from the five subpopulations as part of a single population goal. Gene flow between subpopulations can be managed conservatively or otherwise as long as the genetic health of individual animals and the demographics and genetics of the whole population remain the priority.

MOVING FROM DILEMMA TO SUBSPECIES POLICY

Given that much more powerful tools are available to confirm or refute subspecific status where doubts exist, then policy on subspecies should become less problematic. We would argue that conservation biologists should consider the following in forming a position on subspecies management:

1. Conservation strategies for vertebrates should center on the binomial or full species as the unit of conservation. The species concept represents a tangible unit clearly observable in morphology, behavior and/or genetics and is less subject to artificial construct. Divisions below the species level are problematic since these groups represent a wide variety of evolutionarily unresolved relationships from familial to metapopulation.

2. Naturally occurring patterns of biodiversity that are the result of evolutionary processes are best represented by an operational, typological, phylogenetic species concept. This avoids the introduction of a theoretical framework (such as "potentially interbreeding," Mayr 1969) which is untestable in most circumstances.

3. Identification of species (the "conservation unit") should be done by following a systematics model of character analysis. Characters can include molecular, morphological, behavioral, karyotypic and biogeographical characters. This species definition should recognize observed interbreeding as a primitive character which alone neither supports nor refutes species classification.

4. Armed with a new level of confidence in distinguishing subspecies vs. species, subspecies management should be viewed as an array of opportunities, selection depending upon scientific, political, legal and economic circumstances. While a systematics model describes our conservation unit, population genetics-based strategies should offer appropriate guidance in the maintenance of existing genetic variation within that unit. It remains appropriate for governmental agencies to consider populations for inclusion on endangered species lists.

REFERENCES

Amato, G.D. 1991. Species hybridization and protection of endangered animals. Science 253:250.

Amato, G.D., M.V. Ashley and J. Gatesy. 1993. Molecular evolution in living species of rhinoceros: Implications for conservation. In Ryder, O.A. (ed.) *Rhinoceros Biology and Conservation* 114-122. Zoological Society of San Diego.

Ashley, M.V., D.J. Melnick and D. Western. 1990. Conservation genetics of the black rhinoceros (*Diceros bicornis*) I: Evidence from the mitochondrial DNA of three populations *Conservation Biology* 4:71-77.

Conway, W.G. 1989a. The prospects for sustaining species and their evolution. In Western, D. and M.C. Pearl (eds.) *Conserva for the Twenty-First Century* 199-209. Oxford University Press,

Conway, W.G. 1989b. Altered States: Population viability analysis, planning and caring for wildlife in parks. *Workshop on Analytical Methods for population Viability Analysis and Management.* Smithsonian (Front Royal, VA.).

Cracraft, J. 1983. Species concepts and speciation analysis. *Current Ornithology* 1:159-187.

Cracraft, J. 1989. Speciation and its ontology: the empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. In Otte, D. and J.A. Endler (eds.) *Speciation and its Consequences.* Sinauer Associates, Inc., Sunderland, MA.

Harley, E.H. and C. O'Ryan. 1993. Molecular genetic studies of southern African rhinoceros. In Ryder, O.A. (ed.) *Rhinoceros Biology and Conservation* 101-104. Zoological Society of San Diego.

Mayr, E. 1942. *Systematics and the Origin of Species.* Columbia University Press, NY.

Mayr, E. 1963. *Animal Species and Evolution.* The Belknap Press of the Harvard University Press, Cambridge.

Mayr. E. 1969. The biological meaning of species. *Biological Journal of the Linnean Society* 1:311-320.

Nelson, G.J. and N.I. Platnick. 1981. *Systematics and biogeography: Cladistics and Vicariance.* Columbia University Press, NY.

O'Brien, S.J. and E. Mayr. 1991. Bureaucratic mischief: Recognizing endangered species and subspecies. *Science* 251:1187-1188.

Ryder, O.A. 1986. Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology and Evolution* 1:9-10.

Ryder, O.A.; J.H. Shaw and C.M. Wemmer. 1988. Species, subspecies and *ex situ* conservation. *International Zoo Yearbook* 27:134-140.

Seal, U.S. and E.T. Thorne, M.A. Bogan, SH. Anderson (eds) 1989. *Conservation Biology and the Black-footed Ferret.* Yale University Press, New Haven.

Seal, U.S.; S.A. Ellis; T.J. Foose and A.P. Beyers. 1993. Conservation Assessment and Management Plans (CAMPs) and Global Captive Action Plans (GCAPs). *Captive Breeding Specialist Group News* 4(2):5-10.

Shaw, J.H.; C.M. Wemmer and O.A. Ryder. The conservation of subspecies (unpubl.).

Templeton, A.R. 1986. Coadaptation and outbreeding depression. In Soule, M.E. (ed.) *Conservation Biology: The Science of Scarcity and Diversity*. Sinauer Associates, Inc., Sunderland, MA.

Templeton, A.R.; H. Hemmer; O.A. Ryder; E.G. Cothran; M.E. Soule; N.D. Murray and M. Snyder. 1986. Local adaptation, coadaptation, and population boundaries. *Zoo Biology* 5(2):115-125.

Vogler, A.P. and R. DeSalle. Diagnosing units of conservation management. In press *Conservation Biology*.

Wemmer, C. (ed. in press). Deer: An action plan for the conservation of the Families Cervidae, Moschidae and Asian Tragulidae. IUCN/SSC Deer Specialist Group.

Wharton, D. (ed.) 1985. The SSP subspecies dilemma: A report of an exploratory meeting of the AAZPA Species Survival Plan Subcommittee (SSPC) of the Wildlife Conservation and Management Committee). *AAZPA Annual Conference Proceedings*, 47-54.

Woodruff, D.S. 1989. The problems of conserving genes and species. In Western, D. and M.C. Pearl (eds.) *Conservation for the Twenty-First Century* 76-88. Oxford University Press, N

FOREST ITTO

Tom,
Can you please reply this
letter.
I have a letter for you f
New Delhi. Shall I mail th
letter to you or fax the
contents? Have not op

6-9-93.

Wolfgang Frey
Münchener Str.8
D-82110 Germering
Germany

Mr.Mohd Khan b. Momin Khan
Ibu Pejabat Jbatan Perhutanen
Wisma Sumber Alam
Jalan Stadium, Petra Jaya
93600 Kuching
Sarawak, Malaysia

24.08.93

Dear Mr.Mohd Kahn b. Momin Khan,

One of the recommendations included in your Conservation Action Plan for Asian Rhinos-that is among the very few that are "action plans" not only in name-is to conduct studies to determine whether the now disjunct populations of the Great One-horned Rhinoceros in the Terai and the Brahmaputra Basin constitute evolutionarily significant units justifying preservation as separate entities.

The late Prof.Ulrich informed me that he found differences in the shape of the skulls of either population.

Also the barasinga deer of both areas in question hitherto thought to belong to the same "subspecies",has now been split up in two "subspecies".

I therefore would be very obliged to you for giving me information whether there are plans to remove the animals of Assam or of mixed Assam-Nepal origin from Dudhwa N.P. in order to avoid any mixing in the future.

Your Action Plan doesn't mention the probable occurrence of the Sumatran Rhino in Western Sichuan/China from where reliable reports have been received.

Thank you very much for your kind assistance.

Looking forward to hearing from you.

yours sincerely

Wolfgang Frey