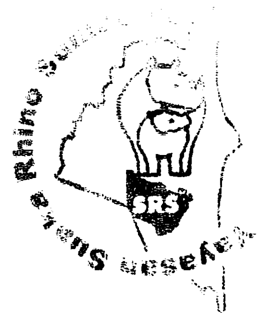
SOLIHIN

Synopsis of Research Project
At The Sumatran Rhino Sanctuary, Way Kambas, Lampung

Individual Identification of The Sumatran Rhinoceros Dicerorhinus sumatrensis (Fischer 1814) In Captive And Wild Population Through Genetic Analysis

By
Dr. Ir. Dedy Duryadi Solihin
Drh. Muhammad Agil, MSc.Agr.
Drh. Marcellus Adi CTR



Center for Life Sciences Study (CLSS)
Bogor Agricultural University
March, 2000

From Nico van Strien's archives
not indexed
project application.

Individual Identification of The Sumatran Rhinoceros *Dicerorhinus sumatrensis* (Fischer 1814) In Captive And Wild Population Through Genetic Analysis

I. INTRODUCTION

Many approach and proposal have been formulated to conserve the Sumatran Rhinoceros in the wild. The starting point for most strategies is an evaluation of animal numbers. The efficiency of measuring of the population size is depended on methode applayed, detailed procedure, accurate and quantitative assessment, and condition of the habitat.

Direct counting methods is not possible to use for this species. Many difficulties have been found. That is why field biologist still have to replayed on indirect methods for population size estimates, especially the analysis of tracks and foot prints, or mathematical guesswork.

Number of the Sumatran Rhinoceros population in the wild have not been known exacly till now. Moreover the sex ratio and individual parentage of the population is also not known. Regarding to the conservation of this species is important to develop an appropriate, accurate, and reliable method for enumeration and individual identification of the animals.

DNA techniques that distinguish between species, populations and individuals are of value to a variety of disciplines. However, the realiability and power of these measures depends not only on the genetic variability in the populations, but also on having a sample size that is large enough to characterize this variation accuratelly. DNA acquired from faeces potentially provides an ideal sampling alternative (Tike[1] et al., 1995; van der Kuyl et al., 1996; Wasser et al., 1997; Frantzen et al., 1998). Faeces is abundant; its collection can be totally noninvasive; and a single gram of faeces contains large quantities of the host's DNA from million of sloughed intestinal mucosal cells (Albough et al., 1992).

Wasser at al., (1997) indicated that the utility of faecal DNA technology for application to the field depends upon the following key issues : (a) the length and copy number of DNA that can be extracted and amplified from faeces; (b) confirmation that the amplified DNA from faeces is identical to that obtained from the same individual's tissue or blood; (c) elimination of sample contamination (e.g. from ingested tissue or hair); (d) prevention of sample degradation in the laboratory or field; and (e) removal of dietary inhibitors.

The overall aims of the project are : (I) to develop non-invasive methodologies for obtaining accurate and reliable information to identify individual Sumatran Rhinoceros; (II) to use the methods to determinereal numbers, sex ratio, and individual parentage of Sumatran Rhinoceros population.

II. OBJECTIVE :

1. To detect genetic variability of the rhinoceros population. revealed by sequens analysis of D-Loop fragment mitochondrial DNA.
2. To detect genetic variability of the rhinoceros population, using microsatellites DNA markers.
3. To determine individual parentage from genetic data, revealed by genetic analysis with microsatellites from faecal samples.
4. To identify individual sex

III. OUT-PUT :

1. Characterization of genetic variability of the rhinoceros population.
2. Determination of parentage of individuals from genetic data.
3. Developing a new technique to detect genetic variability from faecal samples.
4. Proportion of the sex ratio

IV. MATERIALS AND METHODS

A. DNA samples

Genomic DNA is prepared from blood samples & epithel cells in the faecal, using standard protocols involving treatment with SDS and proteinase K, and subsequent phenol / chloroform extraction.

B. Mitochondrial control region analysis

A fragment of the mitochondrial control region is amplified using PCR in 25 ul reaction volume using specific primers. PCR product is used as template for DNA Sequencing (Douzery and Randi, 1997; Kocher et al., 1989; Wood and Phua, 1996).

Analysis of mitochondrial data revealed by nucleotide diversity is used to estimate the genetic diversity within populations. To estimate the genetic distances between populations, the number of net nucleotide substitution between population is calculated.

C. Microsatellite analysis

Two microsatellite loci known to be polymorphic in cattle (*Bos taurus*) such as (AC)n and (TG)n will be used to detect genetic variability of the captive rhineceros (Moore et al., 1992).

Analysis of microsatellites data is doing by measuring genetic diversity within each population such as the mean number of alleles per locus (A), individual genotype, observed heterozygosity (Ho), and heterozygosity expected from Hardy-Weinberg proporsion (He). The likelihood of paternity can be determined with inferential procedures of genealogical structure.

D. Sex Determination

PCR amplification using two primers known to be specific to detect mammalian sex will be used (Aasen and Medrano, 1990). DNA obtained from blood of the individual known sex will be test as sample standard. DNA from faecal samples will be use as a template for determining of the sex of each individu.

V. FACILITIES

(i) Animal facilities and access to animals will use the Sumatran Rhino Sanctuary at Way Kambas, Lampung as center for research on the Sumatran Rhinoceros.

(ii) The genetic analysis (e.g. D-loop mt-DNA, DNA Microsatellites, Molecular sexing, and Sequencing) will be carried out at Center for Life Science Study (CLSS), Bogor Agricultural University.

VI. PERIOD OF RESEARCH PROJECT

Research project will be carried out for 3 years (May 2000 till May 2003).

(i) May 2000 untill May 2001 : Methods development

(ii) May 2001 untill May 2002 : Refinement in the semi captive breeding.

(iii) May 2002 untill May 2003 : Application in the fields

VII. BUDGET

(i) Salary

Three(3) Researchers and Two (2) Technicients each years.....................12 000 US$

(ii) Expendable supplies

Materials for DNA Purification, DNA Amplification, Microsatellites analysis, D-loop mitochondria analysis, Molecular Sex identification, and DNA sequencing

First year ....28 000 US$

Second year .... 25 000 US$

Third year .... 21 000 US$

(iii) Travel

Two times per years ....3 000 US$ each years

Total Budget for three years .... **131 0000 US$**