8.2 ID determination from Rhino Dung DNA: Cunningham & O'Ryan 1998

DETERMINE THE IDENTITY OF INDIVIDUALS FROM THE DUNG OF BLACK RHINOS IN SOUTHERN TANZANIA. THE USE OF HIGHLY POLYMORPHIC DNA MARKERS TO HELP

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INTRODUCTION:

Tanzania has two taxonomic units of black rhino, *Diceros bicornis michaeli* and *D. b. minor*. Recent investigations within the Selous Game Reserve in southern Tanzania have identified four discreet populations of *D. b. minor*. The World Wildlife Fund for Nature (WWF) is to help the Wildlife Division (WD) of the Ministry of Natural Resources and Tourism to try and determine the minimum numbers, distribution and demographic structure of not only this metapopulation but also the remaining populations of rhinos scattered outside the limits of Tanzania's established protected areas. Regrettably, time, funds and the vast areas of heavy evergreen coastal thicket vegetation extending over 500 kM2 (195 M2) in some areas does not permit a comprehensive and protracted ground survey of the remaining rhino populations within the Selous Game Reserve. Consequently the proposed Selous rhino survey methodology is being augmented by the use of faecal DNA analysis to help determine the minimum number of individuals.

Molecular techniques have been used to estimate population substructure, to help identify individuals within animal populations, rates of gene flow and genetic variability. The use of microsatellite DNA (a class of variable markers) allows the detection of highly polymorphic DNA loci which is able to differentiate individuals within a population. This may enable us to calculate the minimum number of individuals in a population if there is a sufficient number of haplotypes. Microsatellite DNA is becoming recognised as the molecular marker of choice for the following reasons: it provides more and better quality data as well as resolution compared to allozymes, and it is more reproducible and gives absolute character descriptions enabling comparison with similar data compiled in other laboratories. This methodology utilizes polymerase chain reaction and therefore only small amounts of starting material, for example biopsies, blood, hair and perhaps feacal samples can provide sufficient DNA.

The primers used to amplify microsatellite loci are directed at unique DNA regions lanking microsatellite repeats, which means that a new microsatellite library has to be made for each new group of animals studied. A black rhinoceros library was constructed in our laboratory and a number of primer sets have been designed.

METHODS

1: LIBRARY CONSTRUCTION AND PRIMER DESIGN:

A black rhinoceros microsatellite library has been constructed (under review), using a standard experimental approach. We have designed 8 primers which should amplify different polymorphic loci. These primers were tested for positive amplification in black rhinoceros and PCR conditions were optimised. DNA samples from a number of southern African populations have been genotyped. Levels of genetic variation within rhino populations have been assessed by examining allelic variation and heterozygosity.

2: FAECAL DNA EXTRACTION:

Dung samples were collected from rhinos in Selous in Tanzania (table 1). DNA was extracted using a published method. Several modifications of this published method are currently being tested. Approximately 100-500 mg of the outer part of the dung bolus was used in the extraction. The DNA from each faecal sample was amplified with the black rhino-specific microsatellite primers. The resultant DNA fragments were separated and the genotypes of each sample was scored.

RESULTS:

1: LIBRARY CONSTRUCTION AND PRIMER DEVELOPMENT:

We isolated 24 (CA) repeat loci after screening 27000 colonies from our library. Amplification primers were designed for 10 loci. Four of the microsatellite loci have yielded positive polymorphic bands, while the primers for the remaining six loci yield multiple amplification bands and so could not be utilised in the analysis.

All of the microsatellite loci tested were polymorphic in black rhinoceros from 3 populations (n=82), with the number of alleles ranging from two alleles to eight alleles per locus, as well as displaying high levels of heterozygosity ($\bar{H}$ = 0.68). There also appears to be sufficient differentiation between the different subspecies - the one individual of the *D. b. longipes* tested had an allele not seen in the other four subspecies of black rhinoceros (*D. b bicornis, D. b minor, D. b michaeli).*

2: ANALYSIS OF FAECAL SAMPLES:

We extracted 25 samples and tested the quality of the extraction by DNA amplification. This first gel resulted of positive amplification of 60% of these initial extractions. These samples resulted in microsatellite products in the appropriate size ranges. We observed 9 different genotypes of these samples (36%) with one primer. We were unable to repeat this amplification to the same specificity in subsequent experiments at the same locus - we did obtain product but these gels displayed multiple non-specific bands which can not be scored with a high degree of confidence. The same variable pattern was obtained when testing primers at the 3 other loci.

Additional experiments performed, suggest that we have co-extracted an inhibitor of DNA amplification. This inhibitor appears to be of plant origin (co-workers in our laboratory that are currently investigating plant systematics support this finding). Our current experiments now are aimed at minimising or removing this inhibitor from our faecal extractions in order to allow us to proceed to the genotyping step

CONCLUSIONS:

1. We have successfully constructed a black rhinoceros specific library. We isolated positive repeat loci and have developed primers that amplify 4 loci reproducibly in 3 populations of southern African rhinoceros.
2. We have demonstrated that DNA can be amplified, albeit not reproducibly, from DNA extracted from black rhino faecal samples.
3. We observed a minimum of 9 unique genotypes (9\25 samples tested) from preliminary data from our initial extractions and genotyping with one of the loci

FUTURE WORK:

We are investigating and will continue to investigate methods that will minimise or reduce the effects of the inhibitor that is being co-extracted with the DNA from the faecal samples. Once this has been achieved we will attempt genotyping of the 35 samples we have received. Additionally, we are continuing to identify more polymorphic repeat loci and develop primers.

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