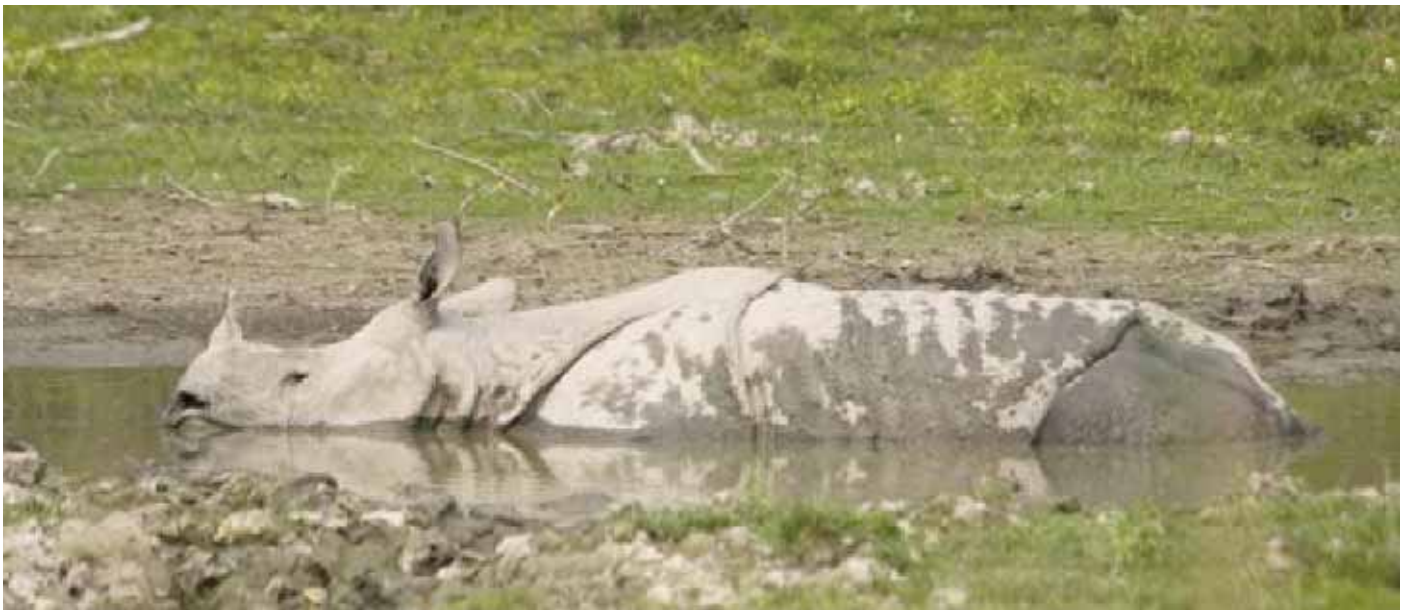
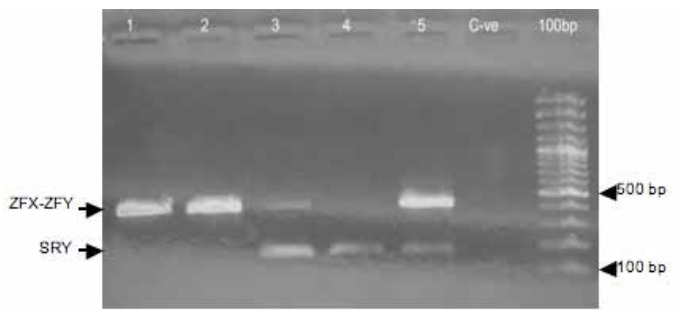


Report Update March 2009: Non-invasive Population Genetic Monitoring of Indian Rhino in Assam: A pilot study to standardise protocols for dung DNA analysis.

Molecular marker based gender identification of Greater one-horned rhinoceros (Rhinoceros unicornis) from dung samples

We have successfully developed a multiplex PCR based marker system for gender identification of rhinoceros from dung DNA samples. Here, we use a combination of Y chromosomal SRY marker with a control of X and Y chromosomal ZFX/ ZFY marker. Using this method, samples from the male can be identified by the presence of both SRY and ZFX/ ZFY PCR products, whereas females are identified by the presence of only ZFX/ZFY products.

For developing this marker system, reference (samples with known gender identity) tissue or dung samples were collected noninvasively, either from dead animals or dung obtained after direct monitoring of defecation from individual rhinoceros during field visits to Kaziranga National Park (KNP), Orang National Park (ONP) and Pobitora Wildlife Sanctuary (PWLS) in Assam.



Above: PCR amplification band pattern of male and female Indian rhinoceros reference DNA. Lane 1 and 2 are female tissue samples, 3 and 4 are male dung samples and 5 is male tissue sample. Lane 6 and 7 are control negative and 100 bp ladder respectively.

Tissues collected from two female and one male rhinoceros and dung collected from two male rhinoceros were used for the work. Apart from the reference samples, 24 rhinoceros dung samples with unknown gender identity were also collected for the study. Of these 24 samples, 10 were collected from KNP, 10 from ONP and four from PWLS.

DNA was extracted from reference tissues and dung samples using DNeasy Blood and Tissue kit (Qiagen, Germany) and QIAamp DNA Stool Mini Kit (Qiagen, Germany) respectively. For amplification of SRY fragment, primers designed from horse SRY sequences (Primers were designed at CREW, Cincinnati Zoo and Botanical Garden) were used for the present investigations. For amplification of ZFX/ZFY marker, P1-5EZ: 5'-ATAATCACATGGAGAGCCACAAGCT-3' and P2-3EZ: 5'-GCACTTCTTTGGTATCTGAGAAAGT-3' (Aasen & Medrano 1990) were used.

All the PCR reactions from dung DNA extracts were run with two positive-controls (One male and one female reference sample) and a negative-control.

Amplified PCR products along with a 100 bp DNA ladder were visualized on an agarose gel containing ethidium bromide. Dung samples with unknown gender identity were screened for three independent PCR replicates.

Male identity was confirmed by the presence of the SRY PCR product in all three reactions and ZFX/ZFY in at least two of the three reactions. Female identity was confirmed by the absence of the SRY band in all three reactions and the presence of ZFX/ZFY PCR band in at least two of the three reactions.

With the method described, we could successfully identify gender for all the reference samples and 18 of the 24 unknown dung samples (12 male and 6 female, a success of 75%) (Borthakur et al. in communication).

Optimisation of PCR amplification from rhino dung DNA samples

We have successfully optimised PCR conditions for 10 polymorphic microsatellite markers developed from Indian rhinoceros (Zchokke et al. 2003) to get amplification from dung DNA samples. So far, we have obtained a PCR success of 80-95% for all the 10 loci tested on 39 dung samples (19 from KNP, 10 from ONP and 10 from PWLS).

We have started microsatellite fragment analysis, which would give us allele size information to be used for calculating probability of identity and heterozygosity, in order to select the minimum number of loci needed for individual identification.

We have successfully amplified mitochondrial DNA marker MDL3/MDL5 (~630 bp), consisting a part of the mitochondrial control region (Fernando et al. 2000) with 90% success from 39 dung samples.

Reference

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Article and photos: Udayan Borthakur

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