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Comparative Genomics Study of *Rhinoceros unicornis* and *Diceros bicornis* via Bioinformatics Approaches: Statistical & Functional

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──────────── ABSTRACT ────────────

Basic agenda of this study is to identify the genomic diversity between the two species on behalf of statistical & functional analysis via computational biology. Genomic approaches are gathering momentum in biology and emerging opportunities lie in the creative use of comparative molecular methods for revealing the processes that influence diversity of wildlife. However, few comparative genomic studies are performed with explicit and specific objectives to aid conservation of wild populations. Here we provide a brief overview of comparative genomics study for *Rhinoceros unicornis* and *Diceros bicornis* via bioinformatics approaches. Because conservation examples are few, we draw on research from other areas to demonstrate how comparing genomic data across wild species may be used to inform the characterisation of conservation units and studies of other molecular properties on behalf of computational biology.

Keywords: *Rhinoceros unicornis*, *Diceros bicornis*, Comparative Genomics, Bioinformatics

INTRODUCTION

ABOUT COMPARATIVE GENOMICS

Conservation genetics has entered the world of genomics (McMohan *et al.* 2014). The number of species with whole-genome sequence data is continually growing (Karolchik *et al.* 2014, Ellegren *et al* 2014), so that more and more endangered taxa are becoming "genome-enabled" (Kohn *et al.* 2014), that is, genome resources are available for them or their close relatives. These new technologies provide researchers with unprecedented levels of data to generate precise estimates of essential population genetic parameters, to examine questions such as the causes and genetic consequences of population decline and fragmentation (Luikar *et al.* 2003, Shafer *et al.* 2015). Most applied conservation genetics research targets issues operating within or amongst populations of the same species (which may be spatially or temporally separated) (Frankham 1995). This level of focus is

often appropriate because anthropogenic threatening processes typically occur over relatively short evolutionary time frames: the scale relevant to population/ species-level processes rather than deeper evolutionary trajectories such as speciation. Nevertheless, there is additional insight to be gained from considering the evolutionary context of threatened species, i.e. by taking a comparative approach across taxa. For example, comparative analyses of species' demographic and life history characteristics have revealed those particular ecological traits that predispose species to high risk of extinction (Purvis *et al.* 2000, Fisher & Owens, 2004). In this paper, we explore how comparative approaches using genomic data may also add value to conservation efforts (Talukdar *et al.* 2008).

ABOUT *RHINOCEROS UNICORNIS*

The Indian rhinoceros (*Rhinoceros unicornis*) is the largest of the three Asian rhino species and has a single horn which can grow upto approximately 60 centimetres in length Like all rhino horns, it is composed of keratin, a protein also found in human hair and nails, rather than bone.

The hairless skin of the Indian rhinoceros is grey or greyish-brown and has many loose folds as well as lumps, known as tubercles, giving this species an armour-plated appearance. The male Indian rhino, which is generally bigger than the female has large, sharp incisors that may be used in fights over females during the breeding season. The Indian rhino is often accompanied by egrets and various species of 'tick birds', including myna, that ride on its back and are thought to feed on parasites between the folds of the rhino's skin. The Indian rhinoceros is classified as Vulnerable (VU) on the IUCN Red List

INDIAN RHINOCEROS DISTRIBUTIONAL RANGE

Previously found throughout the northern Indian subcontinent, the Indian rhinoceros now occurs only in scattered populations in India and Nepal (http://www.arkive.org/black-rhinoceros/diceros-bicornis/)

ABOUT *DICEROS BICORNIS*

The black rhinoceros (*Diceros bicornis*) is the most well known of the five living rhinoceros species, with its aggressive reputation and highly publicised international conservation drive. Black rhinoceros are in fact grey in colour and are distinguished from the other African species (which is also grey) the white rhinoceros (*Ceratotherium simum*), by its pointed, prehensile upper lip; white rhinoceros have square lips. Both African rhinoceros species possess two horns, made from clumped fibres rather than bone, and the taller front horn may be 60 centimetres or longer. *The black rhinoceros is classified as Critically Endangered (CR) on the IUCN Red List.*

BLACK RHINOCEROS DISTRIBUTIONAL RANGE

Once found throughout much of sub-Saharan Africa with the exception of the Congo Basin and other equatorial forest areas of West Africa (4). The recent decimation of the black rhinoceros has restricted the range to fragmented populations, predominately existing in reserves in Kenya, Tanzania, Namibia, Zimbabwe, South Africa, Cameroon, Malawi and Swaziland (4). Four subspecies are recognised in different areas of the species range: the southwestern (*Diceros bicornis bicornis*), western (*D. b. longipes*), eastern (*D. b. michaeli*) and south-central black rhinoceros (*D. b. minor*) respectively (http://www.arkive.org/indian-rhinoceros/rhinoceros-unicornis/).

METHODOLOGY

The genome sequences of *Rhinoceros unicornis* and *Diceros bicornis* were retrieved from National Centre for Biological Information (NCBI) (www.ncbi.nlm.nihgov). Statistical analysis was done via Oligonucleotide Properties Calculator then function study done via mVISTA and kinefold online tools.

RESULTS AND DISCUSSION

Comparative genomics study done on behalf of two modes: Statistical & Functional. Statistical comparative genomics study of *Rhinoceros unicornis* and *Diceros bicornis* was done via online nucleotides properties calculator (Table 1).

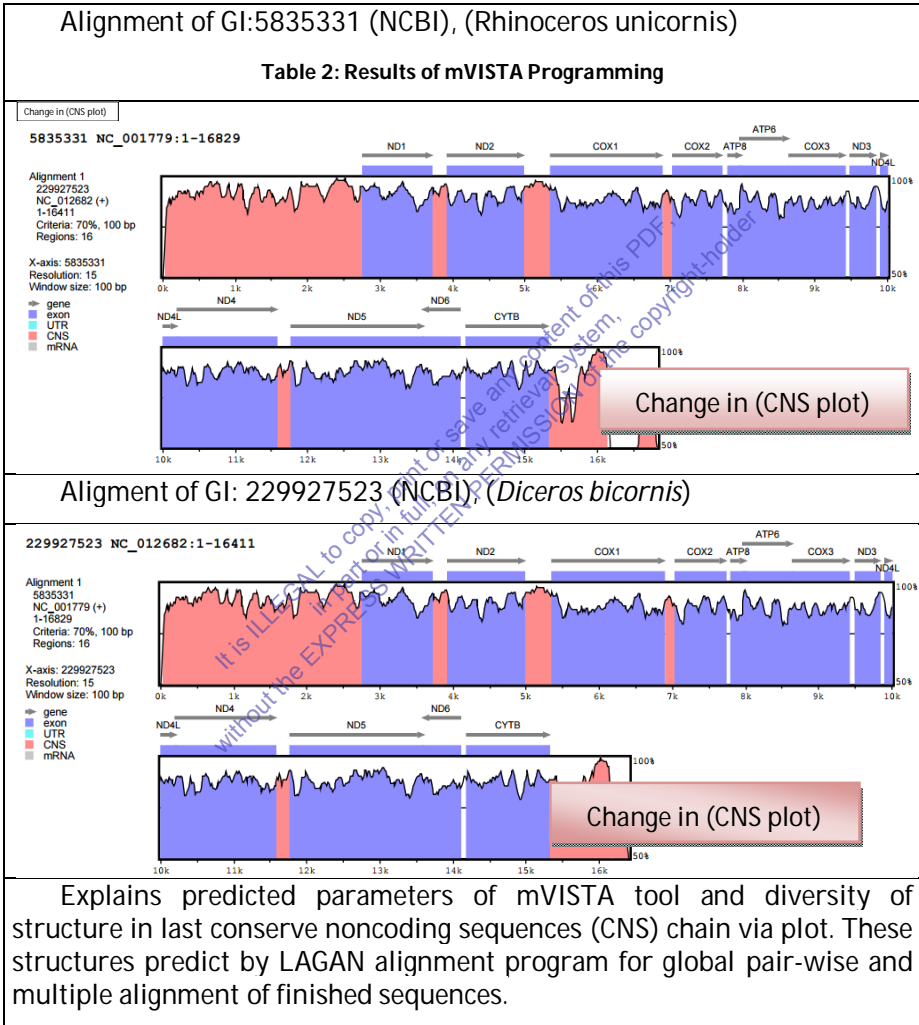**Table 1: Comparative Statistical Analysis of Mitochondrion Complete Genome**

S. No.	Parameters	GI:5835331 (NCBI) (*Rhinoceros unicornis*)	GI: 229927523 (NCBI) (*Diceros bicornis*)
1.	**Physical Constants**		
	Length	16829	16411
	Molecular Weight	5154097.2	5024878.2
	GC content:	40%	41%
2.	**Thermodynamic Constants Conditions: 1 M NaCl at 25°C at pH 7.**		
	deltaG	24918.2 Kcal/mol	24428.5 Kcal/mol
	deltaH	138359.4 Kcal/mol	135297.1 Kcal/mol
	deltas	365788.3 cal/(oK*mol)	357458.8 cal/(oK*mol)
3.	**Melting Temperature (TM) Calculations**		
	°C (Basic)	81.3	81.5
	°C (Salt Adjusted)	95.3	95.7
	°C (Nearest Neighbor)	83.71	83.94

Statistical analysis on behalf of different parameters. Physical constants (Physical analysis of genome sequence), thermodynamic constants (We can assume that the concentration of DNA and the concentration of the DNA-primer complex are equal so this simplifies the equation considerably. If the two strands are in equal concentration, the effective concentration is 0.25 the total concentration of oligonucleotide, melting temperature), Melting

temperature calculations are based on the thermodynamic relationship between entropy, enthalpy, free energy and temperature

STUDY OF FUNCTIONAL COMPARATIVE GENOMICS

This study done via mVISTA and kinefold online tools for Functional diversity between two different species *Rhinoceros unicornis* and *Diceros bicornis*

Alignment of GI:5835331 (NCBI), (Rhinoceros unicornis)

Table 2: Results of mVISTA Programming

Aligment of GI: 229927523 (NCBI), (*Diceros bicornis*)

Explains predicted parameters of mVISTA tool and diversity of structure in last conserve noncoding sequences (CNS) chain via plot. These structures predict by LAGAN alignment program for global pair-wise and multiple alignment of finished sequences.
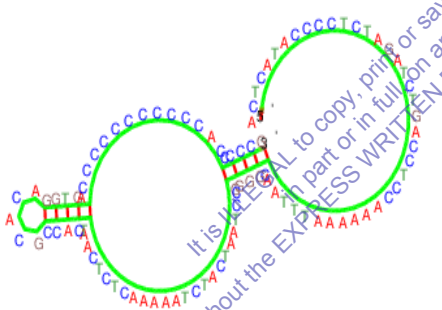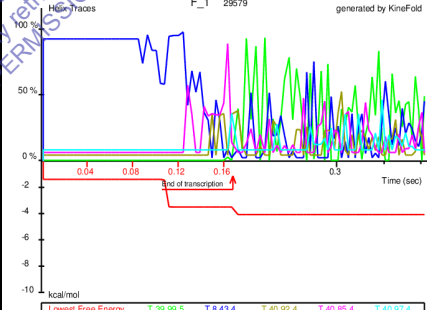
ABOUT MVISTA PROGRAMMING

This is comprehensive suite of programs and databases for comparative analysis of genomic sequences. mVISTA is a set of programs for comparing DNA sequences from two or more species up to megabases long and

visualize these alignments with annotation information (Frazer *et al.* 2004, Mayor *et al.* 2000). mVISTA has a clean output, allowing for easy identification of sequence similarities and differences, and is easily configurable, enabling the visualization of alignments of various lengths at different levels of resolution.

STRUCTURAL ANALYSIS OF FINISHED SEQUENCES

Retrieved last sequence of the genome from NCBI via mitochondrion, complete genome of *Rhinoceros unicornis* and *Diceros bicornis* for diversity of structural analysis via kinefold tool.

Table 3: Minimum Free-energy Structure of Folding Path from Finished Sequences and Helix Tracing Graph for Structural Diversity

Finished_Seq_ GI:5835331 (NCBI), (*Rhinoceros unicornis*)

ACTCATACCCCTCTAGATCTGACCTCCAAAAAATTTAACGGGGCCTAATC
ATCTAAAAACTCTCAATCACCGCACAGGTGACCCCCCCCCGCACCCCCG

Lowest free-energy structure (-4.1kcal/mol)	Helix tracing graph follows the lowest free-energy

Finished_Seq_ GI: 229927523 (NCBI), (Diceros bicornis)

TTATATGTAAAGTCTAATCCCCTTTCCGCACACCTTCCTTTTGATACCAGC
ATGCTACTTTAACCAATAAAATCCACGTAGACTCGTACCCCTCTAGATTTAAT
TTCCAAAAATTCAACAATTCTGATCACCCGCAAACACCTCACACCTCATAGCCC
CCAAAACTCGCCT

Lowest free-energy structure (-0.5 kcal/mol)	Helix tracing graph follows the lowest free energy
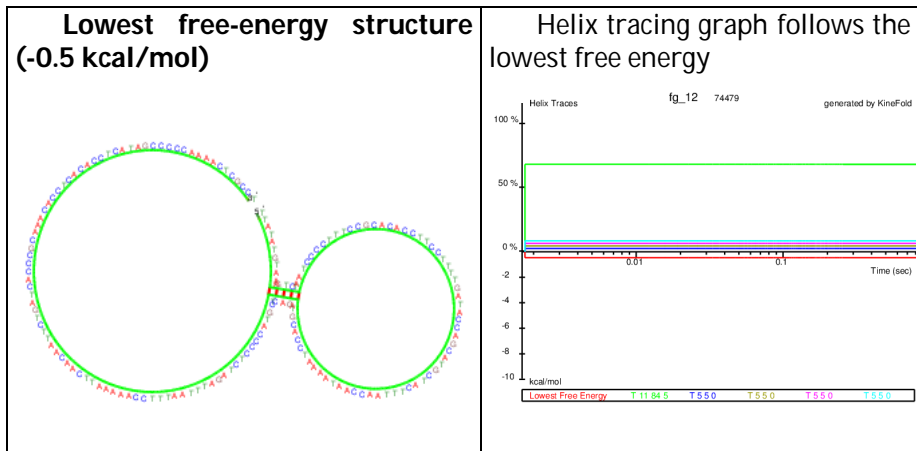
CONCLUSION

Finally in this study we found calculative genomic diversity between two species of Rhinoceros unicornis and Diceros bicornis via computational platform without disturbing wildlife conservation of Rhinoceros. Basic concept for this study is calculating genomic diversity of two species on behalf of statistical & functional analysis. We found diversify statistical and structural calculations as given in table 1, 2, 3 so that is why informatics sciences give us very time saving and validating response and as such bioinformatics is a new revolution in genomic era including wildlife conservation sciences with fully computational validating analysis.

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