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TECHNICAL NOTE

A targeted gene approach to SNP discovery in the White Rhino (*Ceratotherium simum*)

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Abstract We report the characterization of 10 single nucleotide polymorphism (SNP) markers for the White Rhino (*Ceratotherium simum*), based on a targeted gene approach. The polymorphisms of these SNP loci were assessed using a captive population comprising 30 individuals. The minor allele frequency ranged from 0.256 to 0.413 and the observed and expected heterozygosity ranged from 0.05 to 0.37 and from 0.05 to 0.49, respectively. An understanding of genetic population structure is required to effectively formulate strategies for conservation and/or management. These SNP markers could be employed to provide estimates of parameters such as population structure, relatedness and current and historical gene flow.

Keywords SNP · White Rhino · *Ceratotherium simum*

The African White Rhino population has suffered a decline over the past 150 years as a result of overhunting, habitat destruction and poaching (Seror et al. 2002; Florescu et al. 2003). Currently the estimated population of white rhinos comprises 20,170 individuals (Emslie 2011). The trade in rhinoceros horns is a problem in many parts of the world especially in parts of Asia where the rhinoceros horns are used traditionally as material in sculptures or as drug products for medicinal purposes (Hsieh et al. 2003) adding constant pressure on remaining populations. There is thus a real need for markers that can identify the region of origin of rhino products. Genetic diversity and relatedness data for both captive and wild populations also form an important tool in successful reproductive management, population viability assessments and diversity conservation with regards to translocation of animals and establishing breeding programmes (Seror et al. 2002; Harley et al. 2005). The approach described here made use of currently available conserved primer sets designed to amplify from exons across an intervening intron. These CATS primers were designed from other vertebrate genomes to amplify mammalian genes and have been used successfully by Morin et al. (2007) to characterize 18 SNPs for the sperm whale (*Physeter macrocephalus*) and by Li et al. (2009) to describe 51 SNPs in the finless porpoise (*Neophocaena phocaenoides*). The current study is the first to present SNP markers for the white rhino.

Fifteen CATS primers previous described by Aitken et al. (2004) were introduced to discover SNPs from five randomly selected samples. The utilisation of CATS primers allows identification of SNPs in genes of known function so that some genomic information is associated with the identified loci even without prior genomic characterization of the target species (Aitken et al. 2004). The PCR reactions were conducted with Thermo Scientifics' DreamTaq™ according to manufacturer's instructions. Out of the fifteen sets, fourteen amplified successfully and these products were subsequently sequenced for each of the thirty selected samples. Sequencing was performed by Inqaba biotec utilising the ABI Big Dye V3.1 kit (Applied Biosystems) and the ABI 3500XL genetic analyser

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Table 1 Characterization of 10 SNPs in White Rhino (*Ceratotherium simum*)

Locus	SNP name	Sequencing length (bp)	Sequence (5′–3′) (Aitken et al. 2004)	Minor allele frequency	Heterozygosity	
					He	Ho
MGF	MGF-1	820	F-ATCCATTGATGCCTTCAAGG	41.03	0.4902	0.3668
	MGF-2		R-CTGTCATTCCTAAGGGAGCTG	2.56	0.0506	0.0487
ACTC	ACTC-1	875	F-GCCCTGGATTTTGAGAATGAGAT	35.90	0.4662	0.3543
	ACTC-2		R-ACGATCAGCAATACCAGGGTACA	29.49	0.4212	0.3294
	ACTC-3			38.46	0.4795	0.3613
BGN	BGN	647	F-CTCCAAGAACCACCTGGTG	33.75	0.4528	0.3472
			R-TTCAAAGCCACTGTTCTCCAG			
GLUT2	GLUT2F-1	301	F-TGGATGAGTTATGTGAGCAT	41.25	0.4908	0.3672
	GLUT2F-2		R-GACTTTCCTTTGGTTTCTGG	41.25	0.4908	0.3672
KIT	KIT-1	641	F-CCTGTGAAGTGGATGGCACC	13.75	0.2402	0.2091
	KIT-2		R-GCATCCCAGCAAGTCTTCAT	10	0.1823	0.1638

GenBank accession numbers are NCBI_ss#538305377, 538786572-81

F forward primer, *R* reverse primer, *bp* base pairs, *He* expected heterozygosity, *Ho* observed heterozygosity

(Applied Biosystems). GENEPOP version 4.0.10 (Raymond and Rousset 1995) was used to calculate observed (Ho) and expected heterozygosity (He) and to test for genotypic linkage disequilibrium (LD) and deviation from Hardy–Weinberg equilibrium (HWE).

Sequence data was compared between isolates using CLC Bio Main work bench (Denmark). A total of 7523 base pairs of sequence data were generated across the 14 loci for each of the five isolates. Ten SNPs were identified across five of the loci (MGF, ACTC, BGN, GLUT2 and KIT) relating to a discovery rate of one SNP every 752 bp. SNPs were not identified in the following loci: C5, CHY, COL10A1, COL9A1, FES, GHR, HOXD, LPL and WT1. Previous studies utilising CATS loci describe SNP discovery rates of 1SNP/400 bp in chimpanzees (Aitken et al. 2004), 1SNP/540 bp in sperm whale (Morin et al. 2007) and 1SNP/551 bp in finless porpoise (Li et al. 2009). The current study reported a somewhat lower discovery rate which may be attributed to the reported low genetic variation in white rhino populations (Florescu et al. 2003; Harley et al. 2005). The frequencies of the minor alleles ranged from 0.256 to 0.413. The observed and expected heterozygosity ranged from 0.05 to 0.37 and from 0.05 to 0.49, respectively. The BGN marker deviated from Hardy–Weinberg equilibrium which may be attributed to small sample size. Linkage disequilibrium was observed with those SNPs identified in locus ACTC as well as GLUT2. This was expected since these SNPs were in close proximity on the same locus. These markers should be further investigated for applications in other species such as the endangered black rhino (*Diceros bicornis*) (Table 1).

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