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Robust forensic matching of confiscated horns to individual poached African rhinoceros

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Black and white rhinoceros (*Diceros bicornis* and *Ceratotherium simum*) are iconic African species that are classified by the International Union for the Conservation of Nature (IUCN) as Critically Endangered and Near Threatened (http://www.iucnredlist.org/), respectively [1]. At the end of the 19th century, Southern white rhinoceros (*Ceratotherium simum simum*) numbers had declined to fewer than 50 animals in the Hluhluwe-iMfolozi region of the KwaZulu-Natal (KZN) province of South Africa, mainly due to uncontrolled hunting [2,3]. Efforts by the Natal Parks Board facilitated an increase in population to over 20,000 in 2015 through aggressive conservation management [2]. Black rhinoceros (*Diceros bicornis*) populations declined from several hundred thousand in the early 19th century to ~65,000 in 1970 and to ~2,400 by 1995 [1] with subsequent genetic reduction, also due to hunting, land clearances and later poaching [4]. In South Africa, rhinoceros poaching incidents have increased from 13 in 2007 to 1,215 in 2014 [1]. This has occurred despite strict trade bans on rhinoceros products and strict enforcement in recent years.

The significant increase in illegal killing of African rhinoceros and the involvement of transnational organised criminal syndicates in horn trafficking has met with increased law enforcement efforts to apprehend, successfully prosecute and sentence traffickers and poachers with the aim of reducing poaching. In Africa, wildlife rangers, law enforcement officials and genome scientists have instituted a DNA-based individual identification protocol using composite short tandem repeat (STR) genotyping of rhinoceros horns, rhinoceros tissue products and crime scene carcasses to link confiscated evidence to specific poaching incidents for support of criminal investigations. This method has been used extensively and documented in the RhODIS® (Rhinoceros DNA Index System) database of confiscated horn and living rhinoceros genotypes (http://rhodis.co.za), eRhODIS™ applications to collect field and forensic sample data and RhODIS® biospecimen collection kits. These are made available to trained RhODIS® certified officials to fulfill chain of custody requirements providing a pipeline to connect illegally trafficked rhinoceros products to individual poached rhinoceros victims. This study applies a panel of 23 STR (microsatellite) loci to genotype 3,968 individual rhinoceros DNA specimens from distinct white and black rhinoceros populations [5]. We assessed the population genetic structure of these (Supplemental information) and applied them to forensic match analyses of specific DNA profiles in more than 120 criminal cases to date.

Four methods were applied to support forensic matching of confiscated tissue evidence to crime scenes: first, further characterization and optimization of STR panels informative for rhinoceros species; second, development and application of the RhODIS® database containing genotypes and demographic information of more than 20,000 rhinoceros acquisitions; third, analysis of the population genetic structure of white and black rhinoceros species, subspecies and structured populations; and fourth, computation of match probability statistics for specific profiles derived from white and black rhinoceroses. We established a reference database consisting of 3,085 genotypes of white rhinoceros (*C. simum*) and 883 black rhinoceros (*D. bicornis*) sampled since 2010 which provide the basis for robust match probability statistics.

The effects of historic range contractions or expansions, migration, translocation and population fragmentation caused by poaching and habitat reduction on rhinoceros population genetic structure have been reported but are limited [6–8]. Southern white rhinoceros are traditionally considered panmictic and comprising a single subspecies, *C. s. simum*, as a result of the severe founder effect in the late 19th century [2]. Black rhinoceros are generally subdivided into three modern subspecies, *D.b. bicornis*, *D.b. michaeli* and *D.b. minor* [8]. Population structure of white and black rhinoceros based upon three different analyses (Supplemental information) affirmed the partition of white versus black rhinoceros species plus the separation of the three black rhinoceros subspecies. The STRUCTURE algorithm revealed a fine grain distinctiveness between black rhinoceros *D.b. minor* populations from Zimbabwe and KwaZulu-Natal (KZN), South Africa and also indicates that black rhinoceros in the Kruger National Park (KNP) are comprised of a mix of KZN and Zimbabwe rhinoceros as expected, since KNP black rhinoceros founders originated from these two locales [9].

For forensic match applications, we calculated allele frequencies for all polymorphic unlinked loci for white (3,085 genotypes) and black rhinoceros (883 genotypes). These estimates and other STR locus statistics were calculated for each rhinoceros species. Population differentiation (F_{ST}) between white and black rhinoceros subspecies supports the recognition of the Southern white rhinoceros subspecies (*C. s. simum*), and three black rhinoceros subspecies, *D.b. bicornis*, *D.b. michaeli* and *D.b. minor*, with significant partitioning of the Zimbabwe versus KZN *D.b. minor* populations in the present African rhinoceros populations.

Table 1. Summary of nine prosecuted cases of rhinoceros crime.

Match result	Poaching site	Species / subspecies	Match probabilities	Status of case	Nationality
2 horns matched carcass 1 and 1 horn matched carcass 2	KNP, SA	White rhinoceros (*C.s. simum*)	4.20 x 10^{-9}, 2.03 x 10^{-10}	2012/08/23: 29 years and 3 months	Mozambican
Horn matched carcass	Hoedspruit, SA	White rhinoceros (*C.s. simum*)	3.80 x 10^{-8}	2013/03/28: 15 years each	Mozambican and South African
2 horns matched carcass 1 and 1 horn matched carcass 2	Waterberg, SA	White rhinoceros (*C.s. simum*)	1.96 x 10^{-8}, 1.35 x 10^{-8}	2012/11/14: 10 years	Zimbabwean
2 horns matched carcass 1 and 1 horn matched carcass 2	KNP, SA	Black rhinoceros (*D.b. minor*)	4.18 x 10^{-12}, 1.03 x 10^{-12}	2013/08/15: 14 years	Mozambican
The profile from clothing matched carcass	Limpopo, SA	White rhinoceros (*C.s. simum*)	1.19 x 10^{-8}	2015/02/24: 8 years	Zimbabwean and Mozambican
3 horns matched 3 carcasses	ORTIA, SA HiP, SA	White rhinoceros (*C.s. simum*)	8.79 x 10^{-8}, 1.45 x 10^{-9a}, 8.08 x 10^{-8}	2016/11/01: R800 000 fine or 6 years	Chinese
Horn matched blood on carpet	OPC, Kenya	Black rhinoceros (*D.b. michaeli*)	8.98 x 10^{-22}	2017/05/12: 11 years	Kenyan
14 horns with 2 horns matched to a carcass	ENP, Namibia	Black rhinoceros (*D.b. bicornis*)	4.74 x 10^{-13b}	2016/10/30: 14 years	Chinese
6 horns with 2 horns matched to a carcass	KNP, SA	White rhinoceros (*C.s. simum*)	4.55 x 10^{-9}	2014/01/16: 15 months	Vietnamese

Samples were successfully matched using composite STR genotyping with cumulative match probability calculated using a conservative Theta (θ) of 0.1. Details of case with matching evidence items, location of poaching incident, species and subspecies identified, cumulative match probability, status of the case (conviction date: sentence) and the nationalities of the accused are provided for six South African cases and single cases from Kenya, Namibia and Singapore. (KNP – Kruger National Park, SA – South Africa, ORTIA – OR Tambo International Airport, HiP – Hluhluwe-iMfolozi Park, OPC – Ol Pejeta Conservancy, ENP – Etosha National Park). [a] and [b] refer to match probability calculations for specific white and black rhinoceros summarised in Supplemental information.

Over 5,800 rhinoceros crime cases have been submitted to RhODIS® since 2010 and in excess of 120 case reports relating carcass material to evidence items (horn, tissue, blood stains and other confiscated materials) have been provided to investigators. Table 1 summarizes nine of these rhinoceros crime cases which have been concluded in court. These are illustrative of where DNA matches were made and the use of this evidence for prosecution, conviction and sentencing of perpetrators of rhinoceros crimes. Table 1 includes case sample details, species identified and match probability calculated using the RhODIS® reference database. The successful prosecution, conviction and sentencing of suspects in South Africa and other countries affirm the utility of the RhODIS® approach in criminal prosecutions of the perpetrators of illegal rhinoceros trade and provide an international legal precedent for prosecution of rhinoceros crimes using a robust forensic matching of confiscated evidence items to specific wildlife crime scenes.

SUPPLEMENTAL INFORMATION

Supplemental Information including experimental procedures, one figure and one table can be found with this article online at https://doi.org/10.1016/j.cub.2017.11.005.

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