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1 **Contrasting evolutionary history, anthropogenic declines and genetic contact in the**
2 **northern and southern white rhinoceros (*Ceratotherium simum*)**

3

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28

29 **Abstract**

30

31 The white rhinoceros (*Ceratotherium simum*) has a discontinuous African distribution, which is limited by
32 the extent of sub-Saharan grasslands. The southern population (SWR) declined to its lowest number
33 around the turn of the 19th century, but recovered to become the world's most numerous rhinoceros. In
34 contrast, the northern population (NWR) was common during much of the 20th century, declining rapidly
35 since the 1970s, and now only two post-reproductive individuals remain. Despite this species' conservation
36 status, it lacks a genetic assessment of its demographic history. We therefore sampled 232 individuals from
37 extant and museum sources and analysed ten microsatellite loci and the mtDNA control region. Both marker
38 types reliably partitioned the species into SWR and NWR, with moderate nuclear genetic diversity and only
39 three mtDNA haplotypes for the species, including historic samples. We detected ancient interglacial
40 demographic declines in both populations. Both populations may also have been affected by recent
41 declines associated with the colonial expansion for the SWR, and with the much earlier Bantu migrations
42 for the NWR. Finally, we detected post-divergence secondary contact between NWR and SWR, possibly
43 occurring as recently as the last glacial maximum. These results suggest the species was subjected to
44 regular periods of fragmentation and low genetic diversity, which may have been replenished upon
45 secondary contact during glacial periods. The species' current situation thus reflects prehistoric declines
46 that were exacerbated by anthropogenic decline beginning with the rise of late Holocene technological
47 advancement in Africa. Importantly, secondary contact suggests a potentially positive outcome for a hybrid
48 rescue conservation strategy, although further genome wide data are desirable to corroborate these results.

49

50 **Keywords:** white rhinoceros, anthropogenic declines, demographic history, secondary contact,
51 conservation

52

53 **Introduction**

54

55 The white rhinoceros (*Ceratotherium simum*) is the most common of the world's five remaining rhinoceros
56 species. It has borne the brunt of rhinoceros losses during the global acceleration in illegal hunting, which
57 began in 2008 because of increasing demand for horn products in South-East and East Asia. The species
58 is an obligate grazer, thriving historically in two geographically separated grassland areas in sub-Saharan
59 Africa, and has consequently been divided by taxonomists. The southern white rhinoceros (SWR) is
60 endemic to southern Africa, historically occurring in much of the sub-region, south of the Zambezi river,
61 including Namibia, Botswana, Zimbabwe and South Africa (Figure 1A & B, after Rookmaaker & Antoine
62 2012). The northern white rhinoceros (NWR) was endemic to a narrow belt of grassland from west of the
63 Nile River and Albertine Rift, comprising parts of Uganda, South Sudan, the Democratic Republic of the
64 Congo (DRC), Chad and the Central African Republic (Figure 1A & B). The recent histories of both
65 populations are well known, independent, and contrastingly reflect events occurring in Africa and the Middle
66 East since the colonial period from the 18th century (Figure 1C).

67

68 In southern Africa, the northwards spread of colonialism from the Cape of Good Hope resulted in the
69 extermination of the SWR across most of the sub-region (Player and Feely 1960). Even before the turn of
70 the 19th century, the SWR had undergone a population decline so severe that only 100 – 200 individuals
71 remained, restricted to around the confluence of the Black and White Umfolozi Rivers in Zululand (Vaughan-
72 Kirby 1920). However, in 1895 colonial authorities declared the white rhinoceros royal game and proclaimed
73 the area the Umfolozi Junction Reserve (Brooks 2006). With the dedicated conservation action of wildlife
74 authorities in South Africa, this small population increased steadily throughout the 20th century (Figure 1C)
75 to become a conservation success story. The current severe poaching epidemic is threatening to undo
76 these gains, and it is predicted that if present trends continue, the SWR population will start to decline again
77 in 2018 (Ferreira et al. 2015). Efforts to curb recent losses are ineffective with only marginal decreases in
78 poaching rates in 2015 and 2016, with more than 1,000 African rhinoceros killed every year since 2013.
79 Such a population contraction, in the absence of gene flow from other sources, could negatively affect the
80 genetic diversity and evolutionary potential of the SWR through genetic drift.

81

82 The demographic recovery of the SWR is all the more remarkable because the 20th century also brought
83 the near eradication of all other rhinoceros populations across the world. The NWR was still common
84 throughout most of its range at the turn of the 20th century (Roosevelt and Heller 1914, Pitman 1931) and
85 numbers were still relatively high until the 1960s (Emslie and Brooks 1999), when demand for rhino horn,
86 mainly on the Arabian peninsula, precipitated the penultimate poaching epidemic. Political instability and
87 ineffective conservation measures during the ensuing period saw the rapid decline of NWR numbers in the
88 wild (Figure 1C), with the last wild individuals extirpated in Uganda by 1980 (Edroma 1982), in Sudan by
89 1984 (Emslie and Brooks 1999) and finally the population in Garamba National Park, Democratic Republic

90 of the Congo (Hillman-Smith et al. 1986), declared extinct in 2008. The NWR now survives only in captivity,
91 and with two post-reproductive individuals (two females) remaining, its chances of survival look bleak. The
92 imminent extinction of the NWR has sparked several conservation efforts to prevent the loss of what little
93 remains of the population's genetic diversity.

94

95 The plight of the NWR has also precipitated a debate on whether the evolutionary relationship between the
96 two populations could allow for interbreeding and genetic rescue as a conservation strategy (Saragusty et
97 al. 2016), enabling the retention of at least some of the NWR's genetic diversity. The only known NWR-
98 SWR hybrid was a female (Nasi), born in captivity in 1977. Although she survived 30 years in captivity, she
99 never bred, and this has raised questions about the level of reproductive isolation between the two white
100 rhinoceros populations. Although studies have revealed both morphological, behavioural and genetic
101 differences between the SWR and NWR (Groves et al. 2010, Cinková and Policht 2014, Harley et al. 2016),
102 the evolutionary processes giving rise to this differentiation have not been discussed. Several authors have
103 attempted to compare these fossils with extant SWR and/or NWR (Groves 1975, Geraads 2005, 2010), but
104 with limited success due to the scarcity of well-preserved fossil material and difficulty in delimiting
105 species/populations from fossil remains.

106

107 However the fossil record demonstrates clearly that the prehistoric distribution of the white rhinoceros was
108 wider than its recognised historical range. The presence of anatomically modern white rhinoceros in
109 Pleistocene Tanzania, Ethiopia, Libya, Eritrea, and Kenya (Geraads 2011) suggests a demographic history
110 of population contraction and expansion. Fluctuation between cold and arid glacial periods with wet and
111 warm interglacials would have respectively expanded and contracted the grassland biomes on which the
112 white rhinoceros is dependent (Figure 1D). The evolutionary consequences of such climatic fluctuations,
113 especially with regard to demographic isolation, depends on whether climatically driven range expansions
114 allowed NWR and SWR populations to come into demographic secondary contact. The deep divergence
115 between NWR and SWR implied by analysis of mtDNA (0.46 – 0.97 Mya, Harley et al. 2016) is indicative
116 of a prolonged period of demographic isolation between NWR and SWR maternal lineages. However, due
117 to the maternal inheritance of mtDNA, it has a lower effective population size (N_e) than nuclear DNA and
118 its lineages assort more quickly into monophyletic clades. Nuclear markers, especially those that evolve
119 rapidly (such as microsatellites) would be expected to perform reliably in an analysis of demography and
120 isolation by quantifying prehistoric levels of differentiation and gene-flow between populations (Kuhner et
121 al. 1998).

122

123 Here we analysed genetic variation in the white rhinoceros with the aim of more appropriately informing
124 conservation management. We use both nuclear microsatellites and mtDNA to determine levels of genetic
125 variation across a sample of both NWR and SWR populations, and from both wild and captive populations.
126 To estimate the losses in genetic diversity resulting from 20th century population declines, we also measured

127 the genetic diversity of historic (pre-bottleneck) museum material for comparison. Additionally, we also
128 tested the hypothesis that both populations underwent prehistoric demographic size changes, and
129 determined whether the NWR and SWR came into secondary genetic contact after their initial divergence.
130

131 **Methods**

132 Samples and loci

133 Samples were collected from wild (ESM1, Table S1) and captive (ESM1, Table S2) animals for both SWR
134 and NWR. A total of 217 SWR samples (174 wild, 42 captive) and 15 NWR samples (8 wild, 7 captive) were
135 obtained from extant and historic (museum) material representing the entire species range (full details are
136 provided in ESM1, and permit information in ESM1, Table S3). The 5' end of the control region using primers
137 mt15996L (5'-TCCACCATCAGCACCCAAAGC-3') and mt16502H (5'-
138 TTTGATGGCCCTGAAGTAAGAACCA-3') were used to amplify a 477 bp fragment of the control region.
139 Samples were also amplified for 10 microsatellite loci (ESM1, Table S4). The number of markers used in
140 this study is comparable both with the number and identity of markers used in other publication on
141 rhinoceros (Harley et al. 2005; Kotze et al. 2014; Moodley et al. 2017). Markers were selected at random
142 and were developed from a variety of target species (black rhinoceros, SWR and pig). For detailed
143 molecular and quality control methods, see ESM2.

144

145 Genetic diversity

146 We included four previously published mtDNA control region sequences (Genbank accessions AF187836,
147 AF187837, AF187838 and AF187839; Brown and Houlden 2000), as well as seven mitochondrial genomes
148 from wild individuals, three of which were from wild SWR and four from wild NWR prior to that population's
149 extirpation (Harley et al. 2016). Diversity was estimated for all populations separately. Since captive animals
150 were from a variety of zoos and animal parks, we pooled all captive individuals into SWR and NWR groups.
151 For microsatellites, we calculated the mean number of alleles, observed (H_O) and unbiased expected
152 heterozygosity (H_E) using GENETIX (Belkhir et al. 2004). Allelic richness (AR) was computed by resampling
153 to correct for sample size differences among populations. Both AR and inbreeding coefficients (F_{IS})
154 were calculated in FSTAT (Goudet 1995). Mitochondrial DNA diversity for both control region and whole
155 genomes was assessed for levels of polymorphism and haplotype diversity, as well as nucleotide diversity
156 (π), in Arlequin v3.5 (Excoffier and Lischer 2010). Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997) statistics
157 were also calculated in Arlequin to determine whether sequences showed evidence for population size
158 changes.

159

160 Genetic structure

161 Population structure using microsatellite variation was assessed using Bayesian k -means clustering in
162 STRUCTURE (Pritchard et al. 2000). We assumed an admixture model and analysis was run ten times for
163 $k = 1-7$ with each randomly started run consisting of 500,000 Markov Chain Monte Carlo (MCMC) iterations,
164 assuming correlated allele frequencies, discarding the first 100,000 iterations. The optimal k for the
165 microsatellite data was determined as the highest value that was biologically interpretable. MtDNA structure
166 was deduced by constructing a phylogenetic network of control region sequences. We used the median-

167 joining method in Network v5.0.0.1 (Bandelt et al. 1999) with equal weighting on all nodes and using a
168 correction cost algorithm.

169

170 Evolutionary timeframe

171 In order to obtain a time frame for the evolutionary history of the species, we reconstructed a species level
172 maternal phylogeny from the seven mitochondrial genomes sequenced by Harley et al. (2016). We
173 conducted Bayesian phylogenetic dating using BEAST v2.4.3 (Drummond and Rambaut 2007) as this
174 allowed us to parameterise splits in the tree with soft-bounded priors based on known fossil information,
175 using the same mammalian mtDNA genomes and priors described by Harley et al. (2016), except that all
176 five calibration times were used simultaneously in a single analysis. We used the Tamura-Nei model for
177 nucleotide substitution with gamma correction, as deduced by jModelTest v2 (Darriba et al. 2012), placing
178 a relaxed, lognormal prior on the clock rate to account for potential differences in the molecular clock. The
179 analysis was facilitated by a heuristic 100 million-step exploration of the likelihood surface using a Markov
180 chain Monte Carlo simulation, sampling the chain every 100,000 steps and discarding the first 10%.

181

182 Ancient and recent changes in effective population size

183 Ancient demographic change in both SWR ($n = 20$, excluding zoo individuals) and NWR ($n = 15$) populations
184 was inferred using MSVar v.1.3 (Beaumont 1999; Storz & Beaumont 2002), with N_e being the size of a
185 model population that has the same rate of genetic drift as the rhinoceros population of interest. Wide priors
186 were set for all parameter estimates to allow for uncertainties in the data. Three potential scenarios were
187 performed separately for SWR and NWR, assuming different ancestral (N_1) and current (N_0) effective
188 population sizes. These were (i) a stable population ($N_1=N_0$), (ii) a population decline ($N_1>N_0$) and (iii) a
189 population expansion ($N_1<N_0$). Further details of priors and MCMC runs are given in ESM1, Table S5.

190

191 The more recent demographic history of the white rhinoceros, during which humans may have driven
192 population size changes, was investigated through Approximate Bayesian Computation (ABC) simulations
193 (Beaumont et al. 2002). This approach is unlike the likelihood calculations of the data performed by MSVar,
194 but instead simulates a finite set of potential demographic scenarios, which are then compared to the
195 observed data using sets of summary statistics. Demographic histories for SWR and NWR were thus tested
196 independently by exploratory simulations of six scenarios in ABCTOOLBOX v1.1 (Wegmann et al. 2010):
197 a null model, two expansion models, two bottleneck models and a model with two bottlenecks (ESM, Figure
198 S2). Under expansion and bottleneck scenarios we tested whether the timing of the demographic event
199 coincided with sub-Saharan Africa's two most important anthropogenic events - the migration of iron-age,
200 agriculturalist Niger-Congo language speakers (Bantu) into eastern and southern Africa 400-2,000 years
201 ago (ya; Grollemund et al. 2015) and the expansion of colonial-era European influence into the region
202 (present to 400 ya).

203

204 Secondary contact between northern and southern white rhinoceros

205 We also tested for the possibility that NWR and SWR could have come into secondary genetic contact
206 since they diverged from each other. This may have occurred during the late Pleistocene during which the
207 grassland biome would have periodically been continuous between eastern and southern Africa. We
208 therefore built a two-population model that included uni- and bi-directional migration. First, we tested for
209 migration (uni- and bidirectional) at any time during the last glacial period (LGP) of the late Pleistocene
210 (14,000 – 106,000 ya, scenarios 2-4) which followed the end of the Eemian interglacial. We then subdivided
211 the LGP to attempt to differentiate between recent migration during the last glacial maximum (LGM, 14,000
212 – 26,000 ya, scenarios 5-7) and earlier migration during the LGP (26,000 – 106,000 ya, scenarios 8-10).
213 Last, we tested the null hypothesis against a model of ancient (pre-Eemian) migration (130,000 – 500,000
214 ya, scenarios 11-13). For details of model parameterisation see ESM2.

215

216 We were concerned that individuals in our data set could be closely related and we therefore removed all
217 individuals with a relatedness values (r) of 0.3 or higher and reran all single- and two-population ABC
218 simulations.

219

220

221 **Results**

222 A total of 232 white rhinoceros were genotyped at 10 microsatellite loci (ESM3) and 419 bp of the
223 mitochondrial control region (ESM4) was sequenced in 63 individuals. The level of missing data is given as
224 the number and percentage (%) of failed genotypes for the historical and modern data (ESM1, Table S6).
225 Three loci (RHI32A, RH17B and RH17C) for the historic NWR sample showed a high proportion of missing
226 data (> 50%). To determine if missing data at these loci affected the overall observed structure between
227 populations, we reran the Structure analyses for $k = 1-7$ without these loci, and found that there was no
228 change in the overall result.

229

230 Genetic diversity

231 The effect of ascertainment bias was limited in this study as our results were consistent with previous studies
232 using different types of markers in rhinoceros (Merenlender et al. 1989). All populations were found to be
233 in Hardy-Weinberg equilibrium and individual loci were randomly associated (in linkage equilibrium).
234 Nuclear microsatellite genetic diversity was moderate to low, with an average of eight alleles per locus and
235 heterozygosity ranging from 0.48-0.56 (ESM, Table S7). SWR ($H_O = 0.48$) were more diverse than NWR
236 ($H_O = 0.46$), but both populations had lower observed than expected heterozygosity and positive, but not
237 significant, inbreeding coefficients (F_{IS} SWR = 0.09, F_{IS} NWR = 0.33). The extant wild SWR population (six
238 subpopulations) had slightly lower diversity ($H_O = 0.47$) than captive SWR individuals ($H_O = 0.51$). Within
239 the captive SWR there was no difference in genetic diversity between founders and their offspring (zoo-
240 born). In contrast, the wild (museum sampled) NWR population was more diverse than our sample of
241 captive NWR animals, returning a higher mean number of alleles (3.4 vs 2.4) and heterozygosity (0.48 vs
242 0.43). Yet, despite comprising only seven individuals, the captive NWR sample had similar allelic diversity
243 to all sampled wild SWR subpopulations from reserves in South Africa today.

244

245 Mitochondrial genetic diversity was low for both SWR and NWR (ESM2, Table S8), comprising three
246 haplotypes in total, with SWR comprising two haplotypes and NWR just one. Captive SWR contained both
247 haplotypes but had lower nucleotide diversity than wild SWR (0.003 vs 0.005). In both populations, genetic
248 diversity of 19 and 20th century haplotypes were the same as extant levels. We amplified the control region
249 for one ancient SWR individual (shot in South Africa in 1869) which possessed one of the two haplotypes
250 detected in extant SWR populations. Surprisingly, our entire historic NWR sample, containing early 20th
251 century individuals from the three range states in which the NWR was most common, all harboured the
252 same haplotype as NWR population in captivity.

253

254 Genetic structure

255 Both nuclear and mitochondrial markers structured the species into two distinct populations/clades (ESM2,
256 Figure S1), corresponding to SWR and NWR. For microsatellite data, $k = 2$ returned the highest likelihood,
257 with no recent admixture detected between populations (ESM2, Figure S1A). When the data were analysed

258 at higher, less likely, k models, substructure within SWR was revealed with the separation of mainly captive
259 individuals (K4), Mthethomusha Game Reserve (K5) and Origstad and Nkomazi Game Reserves (K6,
260 ESM2, Fig. S4.). However, since we could not assume that our captive sample was taken from a naturally
261 breeding population, only wild SWR were used for subsequent ABC analyses. However, the multilocus
262 profile of the only known SWR-NWR hybrid individual Nasi clearly shows an admixed profile. The
263 mitochondrial control region showed two monophyletic clades, separated by 30 mutational steps (ESM2,
264 Figure S3B). Haplotypes within the SWR were separated by four mutations.

265

266 Evolutionary timeframe

267 Using complete mitochondrial genomes (ESM5), which contained greater levels of within-population
268 polymorphism relative the control region, BEAST analysis inferred a divergence time between the two white
269 rhino lineages at approximately 0.97 million years (Myr), but with a large 95% highest posterior density
270 (HPD) of 0.5-1.5 Myr (ESM2, Figure S1C), with African rhinoceros species (black and white) identified as
271 sister taxa with a most recent common ancestor (MRCA) of 11 Myr (HPD95: 6.4 – 16.0 Myr). Low
272 mitochondrial DNA diversity within each white rhinoceros population resulted in very shallow but similar
273 MRCA times. SWR lineages coalesce to 38,000 years ago (ya, HPD95: 7,400 – 77,400 ya) and NWR
274 mitochondrial genomes shared a common ancestor 34,000 ya (HPD95: 8,800 – 67,000 ya).

275

276 Ancient changes in effective population size

277 While analyses of mtDNA neutrality indicated a history of population contraction for the species, with the
278 majority yielding positive Fu's F_s values, these were not significant for the control region or for mitochondrial
279 genomes (ESM2 Table S8). However, microsatellite data analyses using MSVar revealed that both SWR
280 and NWR have undergone an ancient reduction in effective population size (EMS1, Table S9; ESM2, Figure
281 S5). Independent runs invoking stable, expansion and contraction models all converged to the same
282 posterior values placing current population size (N_0) consistently lower than ancestral population size (N_1 ,
283 EMS1, Table S9; ESM2, Figure S5A & C). The timing of these population contraction events could be dated
284 to the mid-Holocene, between 3,400 – 5,800 ya for SWR and the early Holocene-late Pleistocene (7,000 –
285 29,000 ya) for NWR (EMS2, EMS1, Table S9; ESM2, Figure S5B & D).

286

287 Recent demographic change

288 The null ABC model (Scenario 1; ESM2, Figure S2) of no recent change in population size could be rejected
289 for both SWR and NWR. Instead, highest model support for both populations was for a single decline or
290 bottleneck (Table 1; $BF > 3$). For SWR, the best model selected was that of a population bottleneck during
291 the colonial period (scenario 4 SWR; Table 1) with a modal time for the beginning of the decline of 264
292 years (HPD90 138 – 394 years). In contrast, the best model for NWR was a demographic bottleneck during
293 the time of the Bantu expansion into East Africa (scenario 5 NWR; Table 1), occurring about 1,370 ya
294 (HPD90 518 – 1,869 years).

295

296 Secondary contact and gene flow

297 Combining both SWR and NWR data, parameterised according to the two best single-population scenarios
298 above, we found the marginal densities for all migration models to be higher than the null model of no post-
299 divergence migration (Table 1). Among migration scenarios, bidirectional migration was more likely than
300 any equivalent unidirectional scenario). The highest marginal densities and Bayes factors among
301 bidirectional models were for scenarios set within the LGP (Table 1). Within the LGP, recent LGM
302 secondary contact was the most likely of all tested scenarios, but could not be significantly differentiated
303 from later LGP migration.

304

305 All ABC simulations were also run without closely related individuals ($r > 0.3$). This reduced the sample size
306 of SWR and NWR to 11 and 10 respectively. Nevertheless, all runs returned similar results to those above,
307 with the exception that colonial and Bantu period population bottleneck scenarios could no longer be
308 distinguished from each other for NWR. The results of these additional simulations are provided in ESM1,
309 Tables S10 & S11.

310

311

312

313 **Discussion**

314 We generated molecular data from a sample that included the recent recorded white rhinoceros range, with
315 samples from extant and historic specimens back to the 19th century. We observed much higher allelic
316 variation at microsatellites than for mtDNA, likely reflecting the differences in effective population size
317 between the two markers (approximately 4:1). Levels of microsatellite heterozygosity in white rhinoceros
318 are lower than East, West and southern African black rhinoceros populations (H_E : 0.71 – 0.74, Moodley et
319 al. 2017) but higher than the relatively unmanaged south-western black rhinoceros of Namibia or Angola
320 (H_E : 0.42 – 0.49). We also found that historic levels of NWR diversity were greater than extant levels,
321 demonstrating the negative genetic consequences of the NWR's colonial-era history of hunting and habitat
322 destruction during the latter part of the 20th century. However, for mtDNA, we found that even in colonial
323 times maternal genetic diversity was already as low as it is in extant populations. Therefore, the evolutionary
324 process which reduced maternal variation in both white rhinoceros populations is very likely to have
325 occurred prior the time of sampling in the late 1800's.

326

327 The lower diversity of SWR individuals born in captivity relative to wild-born founders may also indicate a
328 loss of diversity, even in the space of one or two generations. Owing to the increased erosive power of
329 genetic drift in small populations, this effect may increase as time progresses. We therefore suggest an
330 active management plan for captive bred individuals, where multilocus genetic profiles can be used to
331 maintain genetic diversity. Similarly, genetic drift in isolation has differentiated some SWR populations, and
332 more active management between reserves is encouraged to help ameliorate these effects.

333

334 *Structure and evolutionary timeframe*

335 Microsatellite clustering clearly differentiated the white rhinoceros into two distinct populations (ESM2,
336 Figure S1), an observation already made using mtDNA (Groves et al. 2010, Harley et al. 2016) and for the
337 nuclear amelogenin gene (Groves et al. 2010). Both these studies, however, made use of more limited
338 datasets comprising two and seven individuals, respectively. The present study therefore is the first to use
339 large sample sizes and microsatellite markers, and in the case of the NWR the historic sample covered
340 much of the population's range. For maternally inherited mtDNA, dated using five mammalian calibration
341 points, we estimated the divergence of mtDNA lineages at just under a million years, but with wide
342 confidence limits ($\pm 500,000$ years). ABC simulations were not able to narrow this estimate. These wide
343 limits underscore model uncertainty and the wide prior distributions on fossil calibration points.
344 Nevertheless, these divergence estimates provide a general time frame for the initial split between NWR
345 and SWR populations. Genomic analysis will likely be needed to date the divergence of the two white
346 rhinoceros populations more precisely.

347

348 *Ancient population size changes*

349 A coalescent analysis of prehistoric effective population size changes using our microsatellite data allowed
350 us to infer late Pleistocene to mid-Holocene population contractions for both SWR and NWR (ESM2, Figure
351 S4). Since the effective population size inferred through coalescent simulation is a measure of the effect of
352 genetic drift on the genealogical process, reported numbers reflect the minimum number of effective
353 breeders required by the studied population to ameliorate the loss of further diversity through drift. The
354 inferred timeframe largely overlaps with the confidence limits for the coalescence of intra-population mtDNA
355 lineages (ESM2, Figure S1C), which could potentially have occurred as recently as 6,000 – 7,000 ya and
356 as early as 77,000 ya. Although confidence limits on the posterior distribution of these times were large, for
357 SWR, the inferred decline suggests that this population was adversely affected by the grassland contraction
358 that occurred after the LGM. In the NWR, we obtained a signal both a post-LGM (7,000 ya) and a pre-LGM
359 (26,000 – 29,000 ya) population decline, also possibly in response to grassland contraction, highlighting
360 the white rhinoceros' dependence on suitable grassland habitats. These prehistoric population contractions
361 may have been partly responsible for low mtDNA genetic variation detected among colonial-era NWR and
362 SWR samples.

363

364 *Recent human-associated population declines*

365 Microsatellite analysis also allowed us to infer very recent population declines associated with human
366 movements in Africa. We stress that although the timeframes for the recent NWR and SWR bottlenecks
367 were defined to test for an association with known human historical events, our ABC simulations do not
368 provide a causal link between the human activity and white rhinoceros demography. NWR precolonial
369 population decline, may be coincident with the arrival of Bantu speakers from West Africa. Recent
370 reconstructions have inferred that the Bantu expansion proceeded first in a south-easterly direction from
371 Cameroon, avoiding rainforest and taking advantage of a savannah corridor that started to open ~4,000 ya
372 (e.g. Vincens et al. 1998), accelerating ~2,500 ya (Bayon et al. 2012) and leading to colonisation of east
373 Africa and the Great Lakes region around 2,000 ya (Grollemund et al. 2015). While the Bantu were
374 predominantly agriculturalists, utilising grassland habitats on which to grow newly domesticated strains of
375 millet and sorghum, they were also in possession of iron age smelting technology, and thus capable of
376 hunting larger game animals, either directly, or through interactions with and spread of iron-age technology
377 through local hunter-gatherers (e.g. Patin et al. 2014). It is also possible that Bantu speakers associated
378 with people from further afield, either with Arab and South Asian traders via the eastern coast of Africa or
379 with Romans via the Nile Valley. In either case, demand for rhinoceros products, and potentially even live
380 animals, may have helped intensify the decline in effective population size in the NWR observed during this
381 period.

382

383 In comparison, we recovered a clear signal for a more recent human-induced population decline in the
384 SWR, during the occupation of southern Africa by Europeans. This population decline is historically well
385 documented, with the SWR reaching its lowest number of approximately 100 animals over a hundred years

386 after the median time of decline (Figure 2), although the actual time at which the SWR was at its lowest
387 number falls well within the confidence limits of our posterior distribution. Interestingly, although both
388 populations were reduced to low numbers by humans, current effective population size confidence limits
389 did not overlap, showing that the SWR was reduced to significantly lower effective numbers. Although the
390 effective numbers of NWR destroyed by humans was greater, significantly lower effective size for SWR
391 could reflect the more efficient destruction of white rhinoceros by mechanised hunting during the colonial
392 times.

393

394 *Post-divergence gene flow between SWR and NWR*

395 We used two-population ABC analyses to demonstrate that although the NWR may have diverged from the
396 SWR over a million years ago, both populations came into post-divergence secondary contact more
397 recently during the LGP, and potentially even as recently as the LGM. The implications of this finding may
398 prove central in ongoing debates about the specific status of the two white rhinoceros populations, and how
399 best to manage their remaining genetic diversity in the future. The inferred post-divergence gene-flow was
400 likely facilitated by savanna grassland expansions after the Eemian interglacial (115,000 – 130,000 ya), but
401 has ceased completely since the Holocene when NWR and SWR populations declined as their grassland
402 habitat diminished. A potentially continuous distribution of the white rhinoceros is also supported by
403 evidence of its occurrence east of the Nile river from the middle Pleistocene (Clark and Brown 2001), the
404 LGP (Hillman-Smith et al. 1986) and as recently as the Holocene (Gifford et al. 1980). Therefore, the
405 present-day absence of the white rhinoceros east of the Nile River can only be explained by the local
406 extirpation of an East African population during the Holocene contraction, and with repopulation of Uganda,
407 Kenya and Tanzania subsequently attenuated by the flow of the Nile. Taken together, these results suggest
408 that the white rhinoceros has been resilient to population size contractions, which would have subjected
409 local populations to periods of low genetic diversity during interglacial periods, but with diversity being
410 potentially replenished during glacial periods by secondary contact.

411

412 *Conservation implications*

413 The contrasting histories of the northern and southern white rhinoceros have substantial implications for
414 their conservation. Low diversity at both mtDNA and microsatellite loci implies that maintenance of genetic
415 diversity should be a core conservation action for the species. Although the African Rhino Specialist Group
416 advocates a lower limit of 20 founding individuals (Emslie et al. 2009) for new populations, some wild SWR
417 populations like Mthethomusha, Origstad and Nkomazi have already differentiated from the original SWR
418 stock due to management in isolation. Our results suggest not only a minimum number of founders for new
419 populations, but also that microsatellite profiles should be used to select founding individuals from more
420 than one source population. Additionally, low diversity of some populations should be ameliorated by
421 regular and targeted translocations.

422 With most endangered species intensive genetic management of populations would be prohibitively
423 expensive and/or logistically challenging. However, population genetic analysis carried out in forensic
424 context is increasingly being applied in large African mammals, for example in both the forest and savannah
425 elephant to identify the origins of seized animal products (Wasser et al. 2015) and to identify demographic
426 units for conservation management (e.g. Ishida et al. 2018). Forensic studies require large genetic
427 reference databases, thus a large and growing number of white rhinoceros have been routinely genotyped
428 for forensic purposes (Harper et al. 2018), and we advocate making use of this unique genetic resource to
429 aid the management of genetic diversity. With this database, it should be possible to monitor population
430 diversity levels in real time, and select the profiles of immigrant individuals that would maximise population
431 genetic diversity. Since landowners in South Africa are legally obliged to genotype their rhinoceros, it would
432 also be possible, to monitor the breeding success of immigrant individuals as the calves of the next
433 generation are added to the expanding database.

434

435 The situation for the NWR is very different, and here we show that this population is the end-point of a long
436 period of both prehistoric and anthropogenic decline. With only two female individuals remaining, the role
437 of genetics is presently confined to an evaluation of the potential outcomes of hybrid rescue involving the
438 use of SWR genomes. The recent LGP secondary contact is a key result in this context, as it increases the
439 likelihood that hybrid rescue could be positive and that the recently reported NWR/SWR hybrid embryos
440 may provide a viable strategy for conservation of the NWR (Hildebrandt et al. 2018). However drawing such
441 inference could be premature using a handful of genetic markers alone, and for this reason whole
442 resequencing genomic data could be extremely useful in documenting locally adapted regions of the white
443 rhinoceros genome that may be a priority for genetic management in white rhinoceros occupying the
444 northern edge of the species' historic distribution, regardless of the origin of the animals.

445

446 An additional conservation implication of this work is that managed translocation of SWR into some portion
447 of the NWR's historic range might be a viable approach to restore the ecological functionality that this large
448 grazing mammal previously contributed to the northern savannah ecosystem it once occupied (Griffiths et
449 al. 2011), although any such introductions would need to be closely monitored for evidence of a lack of
450 local adaptation, genetic drift and inbreeding.

451

452 **Ethics**

453 Samples collected for this study received ethical approval from Cardiff University and were collected in
454 accordance with the protocols/guidelines of the National Zoological Gardens of South Africa (NZG).
455 Where relevant, animals were handled under the guidelines of the American Society of Mammalogists
456 (ASM; Animal Care and Use Committee, 2011). All required permits are listed in ESM1, Table S3. All
457 museum samples were collected in accordance with the relevant guidelines and regulations of each
458 museum.

459

460 **Electronic Supplementary material**

461 Five files are provided:

462 ESM1, Supplementary Tables

463 ESM2, Methods and Figures

464 ESM3, White rhinoceros microsatellite data

465 ESM4, White rhinoceros control region data

466 ESM5, White rhinoceros complete mitochondrial genomes

467

468 **Data**

469 All DNA sequences generated in this study were submitted to Genbank (accession numbers inserted on
470 final acceptance of paper). Microsatellite data were uploaded to the Dryad database (website given upon
471 final acceptance).

472

473 **Competing interests**

474 The authors have no competing interests.

475

476 **Authors' contributions**

477 YM, IMR, JR, AK, CW and MWB conceived the study. YM, JR, JS, OR, RH, CW performed the fieldwork.
478 IMR, DLD and SS performed the laboratory work. YM, IMR, DLD, SS and MWB conducted the analyses.
479 YM, IMR and MWB wrote the initial draft which was subsequently critically revised and approved by co-
480 authors.

481

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611 **Figure and table captions**

612

613 **Figure 1.** Distribution, phenotype and population trends in the white rhinoceros. A. Historic distribution of
614 northern (NWR) and southern (SWR) white rhinoceros in sub-Saharan Africa (after Rookmaaker & Antoine,
615 2012). B. Northern (left) and southern (right) white rhinoceros females at Ol Pejeta, Kenya. Copyright C.
616 Melzer 2017. C. Population trends of NWR (red) and SWR (blue) through the 20th century. D. Extent of the
617 grassland biome (in red) in Africa during the last glacial maximum (left) when it was continuous from north
618 to south, and at present (right) where it is fragmented.

619

20 **Tables**

21 **Table 1.** Posterior estimates for demographic scenarios within and between Northern and Southern white rhinoceros based on approximate
 22 Bayesian computation. The best model among single and two-population scenarios are indicated in bold.
 23
 24

	<i>Scenario</i>	<i>Scenario Description</i>	<i>Migration prior (kyr)</i>	<i>Posterior Mode (kyr)</i>	<i>Posterior NeM</i>	<i>Marginal Density</i>	<i>P-value</i>	<i>Bayes Factor (BF)</i>
Southern White Rhinoceros	1	Null model (stable population size)	-	-	-	0.01	0.00	-
	2	Expansion during the colonial period	-	-	-	7.49E-05	0.00	0.007
	3	Expansion during the Bantu migrations	-	-	-	2.59E-37	0.00	2.59E-35
	4	Bottleneck during the colonial period	-	-	-	1.68	0.99	168.00
	5	Bottleneck during the Bantu migrations	-	-	-	0.12	0.18	1.2
	6	Two bottlenecks colonial period/Bantu migrations	-	-	-	3.61E-50	0.00	3.61E-48
Northern White Rhinoceros	1	Null model (stable population size)	-	-	-	0.003	0.00	-
	2	Expansion during the colonial period	-	-	-	1.28E-07	0.00	4.27E-05
	3	Expansion during the Bantu migrations	-	-	-	6.45E-35	0.00	2.15E-32
	4	Bottleneck during the colonial period	-	-	-	2.68E-68	0.00	8.93E-66
	5	Bottleneck during the Bantu migrations	-	-	-	0.68	1.00	226.67
	6	Two bottlenecks colonial period/Bantu migrations	-	-	-	1.72E-18	0.00	5.73E-16
Two population models	1	Null model; No migration	-	-	-	739.66	0.56	-
	2	Unidirectional migration (S-N)	Last glacial maximum (14-26)	21.0	5	7,211.49	0.76	9.75
	3	Unidirectional migration (N-S)		18.1	72	2,432.03	0.80	3.29
	4	Bidirectional migration		S-N: 18.1	S-N: 7	19,241.30	0.92	26.01
	5	Unidirectional migration (S-N)		Last glacial period (LGP)	N-S: 21.8	N-S: 112	4,937.74	0.70
	6	Unidirectional migration (N-S)	(14-106)	43.7	7	1,929.91	0.77	2.61
	7	Bidirectional migration		40.0	87	10,643.00	0.86	14.39
	8	Unidirectional migration (S-N)	Early LGP (26-106)	S-N: 43.7	S-N: 7	4,819.14	0.65	6.52
	9	Unidirectional migration (N-S)		N-S: 40.9	N-S: 95	2,099.64	0.83	2.84
	10	Bidirectional migration		72.9	7	9,950.02	0.79	13.45
	11	Unidirectional migration (S-N)	Pre-Eamian interglacial (130-540)	49.4	85	2,377.90	0.65	3.21
	12	Unidirectional migration (N-S)		S-N: 73.7	S-N: 9	1,691.94	0.69	2.29
	13	Bidirectional migration		N-S: 47.0	N-S: 98	5,124.45	0.91	6.93