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1 Northern white rhinoceros pedigree

Figure 1: NWR pedigree highlighting individuals sequenced in this study (in blue box) presumably unrelated, with name, studbook number, ID number, and ploidy number
2 Genetic Divergence

Pair-wise genetic divergence was estimated between all pairs of individuals using sites callable among all individuals, and defined as \((2*\text{homs}+\text{hets})/(2*\text{callable fraction of genome})\), as defined in (Prado-Martinez et al., 2013). Divergence values for all individuals are shown in Supplementary Material Table 1. Calculations were performed on the full set of 9.4 million SNPs.

3 Shared SNP Polymorphism

In order to calculate shared polymorphism between the NWR and SWR, we took the average polymorphism of all possible combinations of the nine NWR
Table 1: Pair-wise genetic divergence for all rhinoceroses included in this study

| Subspecies | ID   | SB 28 | SB 377 | SB 376 | SB 372 | SB 156 | SB 74 | SB 24 | SB 147 | SB 351 | SB 374 | SB 373 | SB 348 | SB 34 |
|------------|------|-------|--------|--------|--------|--------|------|------|--------|--------|--------|--------|--------|------|
| SB 28      | 0    | 0     | 0      | 0      | 0      | 0      | 0    | 0    | 0      | 0      | 0      | 0      | 0      | 0    |
| SB 377     | 0.002202  | 0      | 0      | 0      | 0      | 0      | 0    | 0    | 0      | 0      | 0      | 0      | 0      | 0    |
| SB 376     | 0.001285  | 0.001584663 | 0      | 0      | 0      | 0      | 0    | 0    | 0      | 0      | 0      | 0      | 0      | 0    |
| SB 372     | 0.001375  | 0.0028065797 | 0.00216281 | 0      | 0      | 0      | 0    | 0    | 0      | 0      | 0      | 0      | 0      | 0    |
| SB 156     | 0.000190095 | 0.0001147644 | 0.000111034 | 0.0001308295 | 0      | 0      | 0    | 0    | 0      | 0      | 0      | 0      | 0      | 0    |
| SB 74      | 0.0006290157 | 0.0002747289 | 0.0002786396 | 0.0002680325 | 0.0004756725 | 0      | 0    | 0    | 0      | 0      | 0      | 0      | 0      | 0    |
| SB 24      | 0.001371262 | 0.00049015753 | 0.0001826726 | 0.0001782281 | 0.0001728723 | 0.000163509 | 0    | 0    | 0      | 0      | 0      | 0      | 0      | 0    |
| SB 147     | 0.0013757299 | 0.0004506425 | 0.0004226572 | 0.0001923855 | 0.0001563104 | 0.0005761096 | 0.0006097244 | 0    | 0      | 0      | 0      | 0      | 0      | 0    |
| SB 351     | 0.0019940397 | 0.0022197927 | 0.0020566159 | 0.0015096838 | 0.0004593154 | 0.0006280036 | 0.0003536928 | 0.0003458729 | 0      | 0      | 0      | 0      | 0      | 0    |
| SB 374     | 0.0018568935 | 0.0022159873 | 0.0021562111 | 0.0012253502 | 0.0004202214 | 0.0002685054 | 0.0002954321 | 0.0002804989 | 0.0001285584 | 0      | 0      | 0      | 0      | 0    |
| SB 373     | 0.0020690395 | 0.0023724487 | 0.0021846497 | 0.001194043 | 0.0008237829 | 0.0003680137 | 0.000434705 | 0.0003543035 | 0.0006965531 | 0.0003437573 | 0      | 0      | 0      | 0    |
| SB 348     | 0.0010772812 | 0.0019580472 | 0.0013525782 | 0.0020694494 | 0.0002646699 | 0.0027204395 | 0.0002844941 | 0.0003835173 | 0.0020297771 | 0.0002037643 | 0.0020983745 | 0      | 0      | 0    |
| SB 34      | 0.001022133 | 0.0014089474 | 0.001400131 | 0.001474533 | 0.0016414322 | 0.0045098835 | 0.001651282 | 0.0018329999 | 0.0017460486 | 0.0014700435 | 0.0014909396 | 0.0014120297 | 0      | 0    |

Table 2: Unique number of SNPs estimated for the NWR and SWR individuals studied. Unique number of SNPs are SNPs that only occur in one individual

| Subspecies | ID   | Number of Unique SNPs |
|------------|------|------------------------|
| SWR        | SB 34 | 102537                 |
| SWR        | SB 24 | 106103                 |
| SWR        | SB 147| 124685                 |
| SWR        | SB 156| 97415                  |
| NWR        | SB 28 | 118155                 |
| NWR        | SB 377| 82313                  |
| NWR        | SB 376| 72136                  |
| NWR        | SB 372| 54443                  |
| NWR        | SB 74 | 97750                  |
| NWR        | SB 351| 56155                  |
| NWR        | SB 374| 24510                  |
| NWR        | SB 373| 79715                  |
| NWR        | SB 348| 75545                  |
individuals rarefied down to four individual samples. We then compared this to the four SWR individuals, and determined how many SNPs were polymorphic in both populations, only polymorphic in one population, and how many were fixed differences between the two populations. Calculations were done on the full set of SNPs.

Figure 2: Venn diagram representing shared polymorphism in the NWR and SWR. Colored regions represent SNP loci polymorphic in each population, overlapping area represents loci polymorphic in both samples. Outside shaded area represents the number of loci with fixed differences.

4 Admixture and PCA

After performing 10-fold cross validation on the ADMIXTURE dataset, we found little difference between values of K=1 and K=2 (0.73 and 0.74 respectively), likely due to the recent separation of the two subspecies.
5 Mitochondrial Tree

The final mitochondrial alignment included nine northern and five southern white rhinoceroses, four of the southern sequences obtained from whole genome sequencing and one from Genbank (accession number NC_001808). The control region was excluded from the alignment. Phylogenetic analyses in BEAST 1.6.1 (Drummond and Rambaut, 2007) were performed considering a single partition with a model of sequence evolution corresponding to HKY + G + I, and five partitions as follow: tRNAs, rRNAs, and first, second and third codon sites of the protein coding genes. jModelTest 0.1 (Posada 2008) was used to select models of sequence evolution according to the Akaike Information Criterion: GTR + I (first and third codons, and rRNAs) and TrN (second codon, tRNAs). The monophyly of southern and northern white rhinoceroses was constrained according to a tree inferred using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The Bayesian inference consisted of two concurrent runs with four Markov chains.
(one cold and seven heated chains with a temperature of 0.2), twenty million
generations (sampled every 1,000 generations), and a 10% burn-in. We verified
that potential scale reduction factors were near to 1.0 for all parameters, and
that the average standard deviation of split frequencies was below 0.01. We visu-
alized convergence of runs to stationarity using Tracer version 1.6 (Drummond
and Rambaut, 2007) by verifying no trends in generation versus logL plots. To
estimate the time to the most recent common ancestor (tMRCA) of both rhino
populations, Beast analyses were performed assuming a constant population size
as tree prior and strict molecular clock with a rate of evolution (mean number
of substitutions per site per unit of time) corresponding to 0.0052 (Steiner et al.
in review). The run was performed 108 MCMC generations, with samples taken
every 104 steps, and the first 5 x 104 steps removed as burn-in. Tracer (Drum-
mond and Rambaut, 2007) was employed to analyze the autocorrelation tree
and effective sample size for parameter estimates. The final tree was estimated
in TreeAnnotator v1.8.2 and visualized in FigTree v. 1.4.2.

Species tree inference estimated the time to the most recent common ances-
tor of both white rhinoceros populations around 720 kya (575–862 kya). For the
SWR and NWR populations, the age of the most recent common ancestor was
calculated to 26 kya (8–48 kya) and 13 kya (4–25 kya), respectively, suggest-
ing a relatively recent origin of mitochondrial haplotypes for both subspecies
at the end of the Pleistocene. This is consistent with a previous work on com-
plete white rhinoceros mitochondrial genomes showing the NWR and SWR as
distinct monophyletic clades diverging between 0.46 and 0.97 million years ago
using Bayesian inferences (Harley et al., 2016).

Our species tree inference estimated the mitochondrial divergence time around
720 kya for the two rhino subspecies. As noted above, estimates from both θaδi
and PSMC suggest that these two subspecies diverged less than 80 kya. This
large difference in divergence times could at partially explained by the fact that both $\partial a \partial i$ and PSMC estimate population divergence, and our estimates from the mitochondrial data are for the most recent common ancestor of the two mitochondrial haplotypes, which must occur later than the time of population divergence.

Figure 4: Mitochondrial tree as generated through BEAST

6 Demographic Inference Using $\partial a \partial i$

For the $\partial a \partial i$ analysis, we used 8.7 million SNPs callable in all four southern white rhinoceroses and four of the northern white rhinoceroses. We used the folded frequency spectrum, which considers only minor allele frequencies. We fit to the data a series of increasing complexity models. One model included a split into two populations, followed by exponential growth. The second set of models constrained the northern and southern population sizes to fractions of the ancestral population size, followed by either exponential growth or a growth model similar to that used by (Gutenkunst et al., 2009). Results are presented
in Supplemental Table 3. We include both the estimates scaled to $\theta$, as reported by $\partial a \partial i$, as well as estimates in natural units when appropriate.

Table 3: Results from the three tested $\partial a \partial i$ models. $T_{split}$ represents estimated split from ancestral population; $N_a$ is ancestral population size; $n_1$ and $n_1$ the is size of the NWR and SWR populations at the time of the split from the ancestral population; for the fractional models, $s$ is the fraction of the ancestral population which becomes the NWR at the split, $m_{NS}$ and $m_{SN}$ is the north-south and south-north migration rate.

| Model       | $T_{split}$ | likelihood theta | theta | $n_1$ | $n_2$ | $n_1F$ | $n_2F$ | $m_{NS}$ | $m_{SN}$ | $T_{split}$ |
|-------------|-------------|------------------|-------|-------|-------|--------|--------|-----------|-----------|-------------|
| Null Split  | -870969.10  | 3006281.45       | 2165524.44 | 253.38 | 4912.69 | 3486.96 | 1975.91 | 2351.05  | 193799.45 |
| Split       | -880133.70  | 3614588.14       | 2617626.71 | 4439.95 | 706.14  | 1783.89 | 1390.41 | 83923.32 | 2031.46   |
| Fraction    | -74245.30   | 3716924.37       | 4341.20  | 470.51 | 1803.29 | 9233.60 | 54.96  | 18550.98 | 9212.84   |

7 Inbreeding

We calculated the number of regions that could be considered a run of homozygosity (ROH), which is considered a good measure of inbreeding (McQuillan et al., 2008). We choose a window size of 1 Mbp according to (Pemberton et al., 2012), which identified regions of homozygosity smaller than 0.5 Mbp as the result of background relatedness, and regions larger than 1.6 Mbp as the result of recent parental relatedness.

We determined shared runs of homozygosity by calculating ROH shared by two or more rhinoceroses in each population. To compare the NWR to the SWR, we resampled all possible four rhinoceroses combinations from the nine NWR, and determined how often a ROH was shared by two or more rhinoceroses in a 4 individual sample.
Supplementary Material Figure 5. Runs of homozygosity shared by two or more individuals in each population, for lengths of 1, 5, 10, 15, 20, 25, and 30 Mbp. Error bars represent the standard deviation from resampling all possible four rhinoceroses combinations in the NWR.

Figure 5: Runs of homozygosity shared by two or more individuals in each population, for lengths of 1, 5, 10, 15, 20, 25, and 30 Mbp. Error bars represent the standard deviation from resampling all possible four rhinoceroses combinations in the NWR.

8 Selection

Figure 6: Tajima’s D values for all scaffolds in the southern white rhinoceros genome. Red lines represent the boundaries of the 1% quantiles.
Table 4: List of all genes containing outliers coding SNPs as identified by the Tajimas D test.

| Gene description                                      | Ensembl ID     | SNP count | HGNC symbol | Gene Ontology ID                                      |
|-------------------------------------------------------|----------------|-----------|-------------|-------------------------------------------------------|
| interferon beta 1                                     | ENSCAF00000001653 | 19        | IFNB1       | GO:0002250, GO:0002281, GO:0002312, GO:0005125, GO:0005126, GO:0005132, GO:0005576, GO:0005615, GO:0006952, GO:0006959, GO:0007166, GO:0007596, GO:0008811, GO:0009615, GO:0030101, GO:0030183, GO:0033141, GO:0035458, GO:0042100, GO:0042742, GO:0043330, GO:0045071, GO:0045089, GO:0045343, GO:0045581, GO:0045944, GO:0051607, GO:0060337, GO:0060338, GO:0071359, GO:0071360, GO:0071549, GO:0098586, GO:2000552, GO:2001235 |
| protease, serine 58                                   | ENSCAF00000003823 | 31        | PRSS8       | GO:0004252, GO:0005576, GO:0006508, GO:0008233, GO:0008236, GO:0016787 |
| potassium channel tetramerization domain containing 12| ENSCAF00000005068 | 1         | KCTD12      | GO:0003723, GO:0005886, GO:0016020, GO:0030054, GO:0042734, GO:0042802, GO:0045202, GO:0045211, GO:0051260, GO:0070062 |
| Gene Name | ENSCAF00000005072 | 1 | FBXL3 | GO:0000151, GO:0000209, GO:00004842, GO:00005515, GO:00005634, GO:00005737, GO:00005829, GO:00014567, GO:00016604, GO:00019005, GO:00031146, GO:00031648, GO:00042752, GO:00043153, GO:00043161, GO:00043687, GO:00048511 |
|-----------|--------------------|---|--------|---------------------------------|
| ER membrane protein complex subunit 3 | ENSCAF00000005202 | 24 | EMC3 | GO:00003674, GO:00008150, GO:00016020, GO:00016021, GO:00034975, GO:00072546 |
| olfactory receptor family 4 subfamily E member 2 | ENSCAF00000005679 | 2 | OR4E2 | GO:00004871, GO:00004888, GO:00004930, GO:00004984, GO:00005886, GO:00007165, GO:00007186, GO:00007608, GO:00016020, GO:00016021, GO:00050896, GO:00050911 |
| olfactory receptor family 52 subfamily N member 2 | ENSCAF00000006321 | 2 | OR52N2 | GO:00004871, GO:00004930, GO:00004984, GO:00005886, GO:00007165, GO:00007186, GO:00007608, GO:00016020, GO:00016021, GO:00050896, GO:00050911 |
| heat shock protein family H (Hsp110) member 1 | ENSCAF00000006538 | 14 | HSPH1 | GO:0000166, GO:0000774, GO:00005515, GO:00005524, GO:00005576, GO:00005634, GO:00005654, GO:00005737, GO:00005829, GO:00005874, GO:00006898, GO:00006986, GO:00043014, GO:00043234, GO:00045345, GO:00045944, GO:00051065, GO:00051135, GO:00061098, GO:00070062, GO:00071682, GO:19003034, GO:1903748, GO:1903751, GO:1903753, GO:2001234 |
| olfactory receptor family 10 subfamily V member 1 | ENSCAF00000007561 | 1 | OR10V1 | GO:00004871, GO:00004930, GO:00004984, GO:00004984, GO:00005886, GO:00007165, GO:00007186, GO:00007608, GO:00016020, GO:00016021, GO:00050896, GO:00050911 |
| olfactory receptor family 5 subfamily G member 3 | ENSCAF00000007988 | 8 | OR5G3 | GO:00003950, GO:00003950, GO:00005557, GO:00005886, GO:00005887, GO:00006471, GO:00006501, GO:00006620, GO:00016740, GO:00016757, GO:00031225, GO:00070062 |
| ADP-riboseyltransferase 3 | ENSCAF00000008589 | 4 | ART3 | GO:00003950, GO:00003950, GO:00005557, GO:00005886, GO:00005887, GO:00006471, GO:00006501, GO:00006620, GO:00016740, GO:00016757, GO:00031225, GO:00070062 |
| Gene Name                                    | Accession Number       | Protein Name                   | GO Terms                                      |
|----------------------------------------------|------------------------|--------------------------------|-----------------------------------------------|
| NADH:ubiquinone oxidoreductase subunit B4    | ENSCAF00000011204      | NDUFB4                         | GO:0005739, GO:0005743, GO:0006712, GO:0006713 |
| olfactory receptor family 2 subfamily W member 1 | ENSCAF000000012133    | OR2W1                          | GO:0004871, GO:0004930, GO:0004984, GO:0005886 |
| galectin 12                                  | ENSCAF000000015091     | LGALS12                        | GO:0005634, GO:0005739, GO:0006915, GO:0007165 |
| acyl-CoA synthetase medium chain family member 3 | ENSCAF000000017952     | ACSM3                          | GO:0000166, GO:0003674, GO:0003824, GO:0003996 |
| alpha-2-macroglobulin                        | ENSCAF000000025567     | A2M                            | GO:0001869, GO:0002020, GO:0002576, GO:0004866 |
| olfactory receptor family 8 subfamily K member 1 | ENSCAF000000028823     | OR8K1                          | GO:0004871, GO:0004930, GO:0004984, GO:0005886 |


| Protein Name                                   | Accession   | Gene Symbol | GO Terms                                      |
|-----------------------------------------------|-------------|-------------|-----------------------------------------------|
| Immunoglobulin heavy constant mu             | ENSCAF000800030258 | IGHM        | GO:0002250, GO:0002376, GO:0003697, GO:0003823, GO:0005515, GO:0005576, GO:0005615, GO:0005886, GO:0006911, GO:0006958, GO:0009986, GO:0016020, GO:0016021, GO:0019731, GO:0031210, GO:0034897, GO:0042834, GO:0045087, GO:0050829, GO:0050853, GO:0050871, GO:0050900, GO:0070062, GO:0071756, GO:0071757, GO:0072562 |
| Olfactory receptor family 5 subfamily M member 3 | ENSCAF000800032727 | OR5M3       | GO:0004871, GO:0004930, GO:0004984, GO:0005549, GO:0005886, GO:0007165, GO:0007186, GO:0007608, GO:0016020, GO:0016021, GO:0050896, GO:0050911 |
| Olfactory receptor family 56 subfamily A member 1 | ENSBTAG000800000368 | OR56A1      | GO:0001559, GO:0001963, GO:0004871, GO:0004984, GO:0005886, GO:0007165, GO:0007186, GO:0007194, GO:0007195, GO:0007608, GO:0009636, GO:0014059, GO:0016020, GO:0016021, GO:0030672, GO:0035240, GO:0042493, GO:0043266, GO:0048148, GO:0048149, GO:0050896, GO:0050911, GO:0051481, GO:0051967, GO:0060158, GO:1901386 |
| Tumor protein D52 like 3                      | ENSBTAG000800011160 | TPD52L3     | GO:0005515, GO:0005576, GO:0016020, GO:0016021, GO:0043462, GO:0044339, GO:0045609, GO:0045668, GO:0045672, GO:0045780, GO:0051480, GO:0090900 |
| Transmembrane protein 64                      | ENSBTAG000800011268 | TMEM64      | GO:00005509, GO:00005515, GO:00005886, GO:0016020, GO:0016021, GO:0017121, GO:0017124, GO:0019899, GO:0042609, GO:0070062, GO:0071222 |
| Phospholipid scramblase 4                     | ENSBTAG0008000011966 | PLC5R4      | GO:00005509, GO:00005515, GO:00005886, GO:0016020, GO:0016021, GO:0017121, GO:0017124, GO:0019899, GO:0042609, GO:0070062, GO:0071222 |
| Gene Name                        | Gene ID                              | Chromosome | Gene Family | Subfamily | Scaffold ID | Transcript ID | Description                                      |
|---------------------------------|--------------------------------------|------------|-------------|-----------|-------------|--------------|-------------------------------------------------|
| Centrin 1                       | ENSBTAG00000012320                   | 2          | CETN1       |           |             |              | GO:0000922, GO:0005509, GO:0005515, GO:0005685, GO:0005737, GO:0005814, GO:0005815, GO:0007049, GO:0008017, GO:0031683, GO:0032399, GO:0032795, GO:0034605, GO:0046872, GO:0051301, GO:0005509, GO:0007214, GO:0008277, GO:0032228, GO:0046872, GO:0050966, GO:0004871, GO:0004930, GO:0004984, GO:0005886, GO:0007165, GO:0007186, GO:0007608, GO:0016020, GO:0016021, GO:0050896, GO:0050911 |
| Olfactory receptor family 2     | ENSBTAG00000032670                   | 47         | OR2S2       |           |             |              | GO:0004871, GO:0004930, GO:0004984, GO:0005886, GO:0007165, GO:0007186, GO:0007608, GO:0016020, GO:0016021, GO:0050896, GO:0050911 |
| Olfactory receptor family 2     | ENSBTAG00000038518                   | 37         | OR2D3       |           |             |              | GO:0004871, GO:0004930, GO:0004984, GO:0005886, GO:0007165, GO:0007186, GO:0007608, GO:0016020, GO:0016021, GO:0050896, GO:0050911 |
| G protein-coupled receptor 39   | ENSBTAG00000047036                   | 7          | GPR39       |           |             |              | GO:0004871, GO:0004930, GO:0005886, GO:0007165, GO:0007186, GO:0016020, GO:0016021, GO:0046872 |
| Olfactory receptor family 56    | ENSBTAG00000047176                   | 11         | OR56B4      |           |             |              | GO:0004871, GO:0004930, GO:0004984, GO:0005886, GO:0007165, GO:0007186, GO:0007608, GO:0016020, GO:0016021, GO:0050896, GO:0050911 |

9 Identification of the X chromosome

In order to identify scaffolds in the rhino genome corresponding to the X chromosome, we first attempted to BLAST all scaffolds against the horse X chromosome. However, a large number of scaffolds contained sequences highly similar to the horse X. We also determined the location of all genes occurring on the horse X chromosome using the UCSC Table Browser, and then identified the homologous genes in the rhino genome using biomaRt (Durinck et al., 2005) R (R Core Team, 2016). We identified 45 scaffolds in the white rhinoceros genome.
with homologs of genes found in the horse X chromosome. These scaffolds represent 17% of the total size of the rhino genome. Due to difficulties in identifying X chromosome genomic regions in the rhino genomes, we did not filter or exclude the scaffolds identified as X chromosome in any of the genomic analyses. These results are shown in Table 5.

Table 5: Scaffolds in the southern white rhino genome with homologs on the horse X chromosome. The table contains the scaffold ID, the size of the scaffold, and the number of genes associated with the horse X chromosome found on the scaffold.

| Scaffold | Size   | Count | Scaffold | Size   | Count |
|----------|--------|-------|----------|--------|-------|
| JH767773 | 18099069 | 103   | JH767905 | 395792 | 3     |
| JH767774 | 17977685 | 76    | JH767914 | 275042 | 3     |
| JH767807 | 9651078  | 52    | JH768001 | 39903  | 3     |
| JH767790 | 12399428 | 45    | JH767733 | 39176111 | 2 |
| JH767876 | 1268962  | 43    | JH767755 | 25004955 | 2 |
| JH767813 | 8371206  | 38    | JH767771 | 18387346 | 2 |
| JH767823 | 5930997  | 24    | JH767844 | 3258986 | 2     |
| JH767846 | 3216515  | 23    | JH767857 | 2430860 | 2     |
| JH767870 | 1596305  | 20    | JH767863 | 1968646 | 2     |
| JH767785 | 14444393 | 17    | JH767871 | 1169348 | 2     |
| JH767819 | 7485276  | 15    | JH767936 | 137788  | 2     |
| JH767831 | 4435101  | 15    | AKZM01054956 | 6131 | 1     |
| JH767838 | 3782617  | 13    | JH767732 | 44411711 | 1 |
| JH767841 | 3625529  | 12    | JH767739 | 33267342 | 1     |
| JH767890 | 699797   | 11    | JH767741 | 32302253 | 1     |
| JH767897 | 472045   | 9     | JH767745 | 29576853 | 1     |
| JH767907 | 344087   | 7     | JH767752 | 26277727 | 1     |
| JH767882 | 860638   | 6     | JH767791 | 12347721 | 1     |
| JH767884 | 873626   | 6     | JH767796 | 11665060 | 1     |
| JH767866 | 1829894  | 5     | JH767837 | 4025681  | 1     |
| JH767910 | 334094   | 4     | JH767899 | 463069  | 1     |
| JH767852 | 2826735  | 3     | JH767917 | 290250  | 1     |
| JH767892 | 532191   | 3     |         |        |       |
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