

## **Supplementary data:**

### **Origin and phylogeography of African savannah elephants (*Loxodonta africana*) in Kruger and nearby parks in Southern Africa**

Authors: Alida de Flamingh<sup>1,2</sup>, Alfred L. Roca<sup>2,3,4</sup>, Rudi J. van Aarde<sup>1</sup>

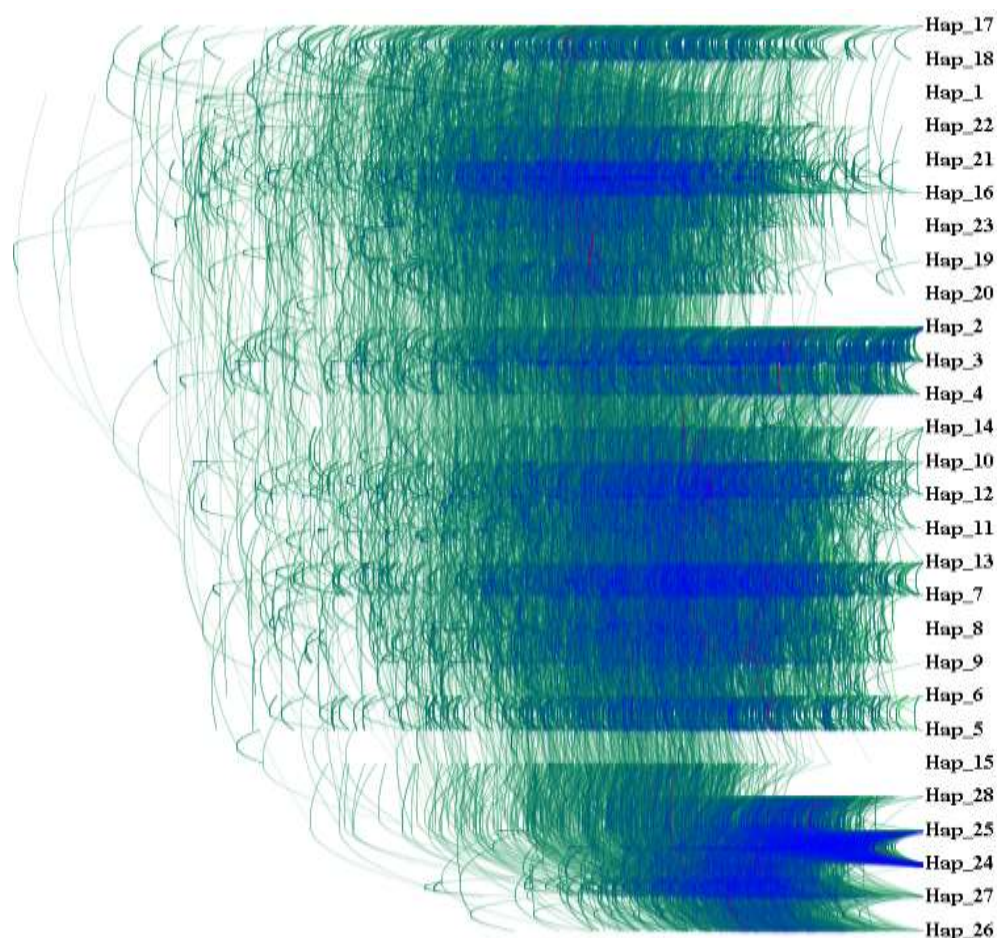
Corresponding author: Alida de Flamingh, [adeflamingh@gmail.com](mailto:adeflamingh@gmail.com)

<sup>1</sup> Conservation Ecology Research Unit, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa

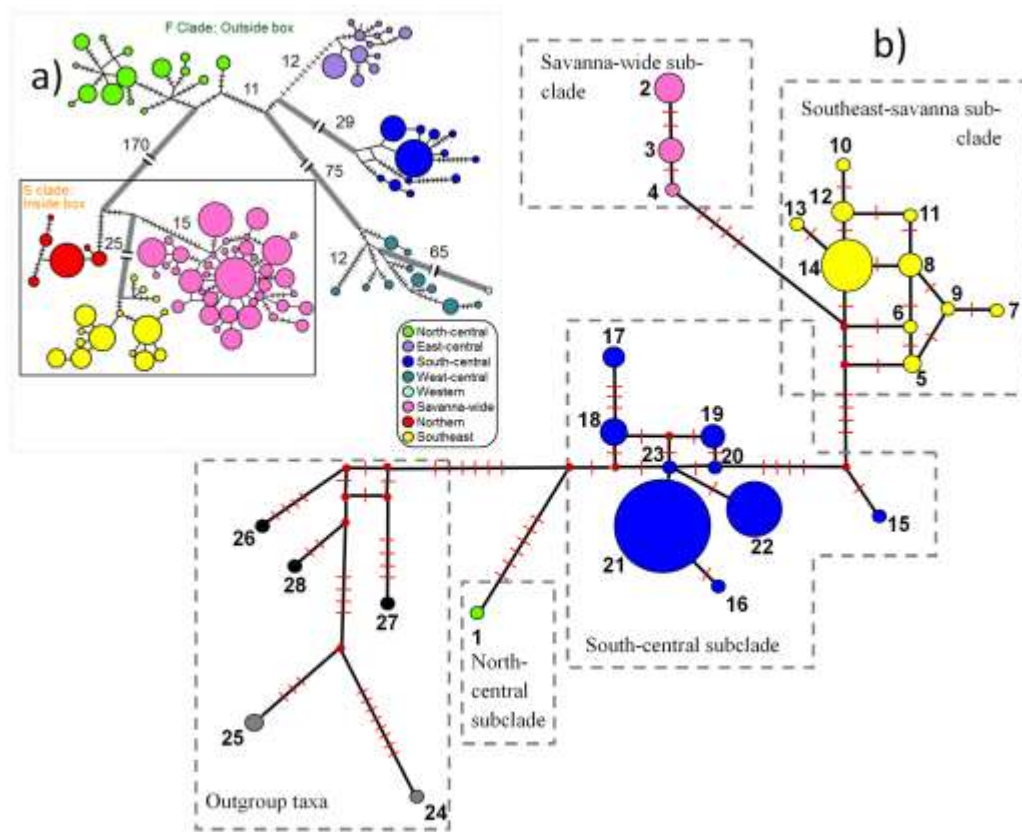
<sup>2</sup> Program in Ecology, Evolution and Conservation Biology, School of Integrative Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA

<sup>3</sup> Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA

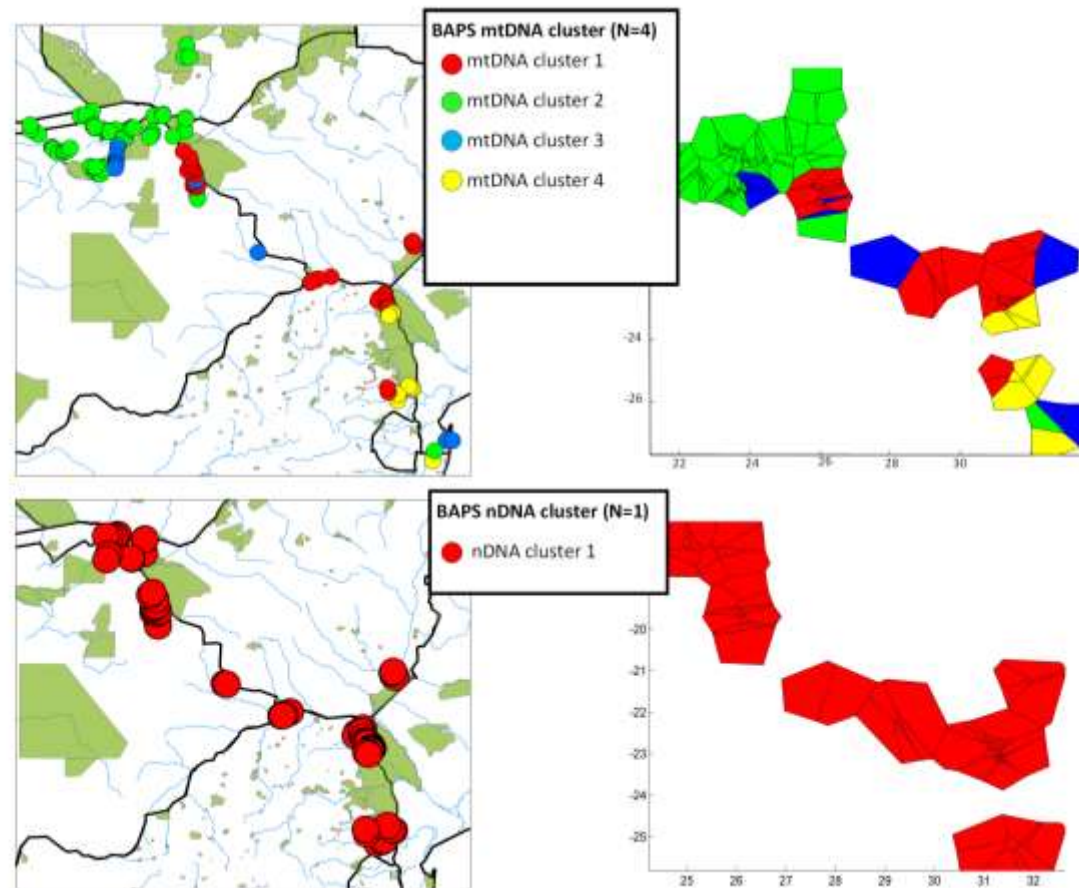
<sup>4</sup> Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA



**Supplementary Figure 1.** A cloudogram of all tree topologies (green) and consensus tree topologies (blue) produced by Bayesian analysis (1000 topologies, of which 20% [200 topologies] are excluded as “burn-in”) illustrates uncertainty in relationships among haplotypes.



**Supplementary Figure 2.** Mitochondrial DNA sub-clades as reported (a) by Ishida et al (2013) and (b) in Figure 2 of this study. Branch length is proportional to the number of mutational differences (indicated as cross-hatches) between haplotypes, circle size is proportionate to the number of individuals carrying a haplotype for each of the networks, respectively. Colors indicate similar sub-clades across networks. Haplotype 15 most closely matched previously reported sequences for the South-central subclade, but was also distinctive from other haplotypes within that subclade. The figure in panel (a) is reproduced under the creative commons license CC BY-NC 3.0 and with permission from original authors.



**Supplementary Figure 3.** Bayesian Analysis of Population Structure (BAPS) clustering analysis the mtDNA dataset to be best partitioned into four populations and the nDNA dataset to conform to a single population. Figures on the left and right panels respectively show the geographic distribution of clusters based on sampling locations and as Voronoi tessellations for mtDNA (top) and nDNA (bottom).

**Supplementary table 1.** Marker names, profiles and characteristics<sup>4</sup> of nine microsatellite loci used in this study.

Marker name	Locus name	Repeat motif	Size (bp)	Ta-°C	Number of alleles	Ho	He
LaT08 <sup>2</sup>	1	(TAGA)16	166–234	56	13	0.774	0.861
Lat13 <sup>2</sup>	2	(CATC)21	234–262	56	7	0.650	0.769
Lat17 <sup>2</sup>	3	(GGAT)15... (GGAT)	323–355	56	6	0.737	0.779
Lat24 <sup>1</sup>	4	(GGAT)22	211–231	56	8	0.694	0.838
FH1 <sup>1</sup>	5	(CA)12	81	55	5	0.612	0.661
FH39 <sup>1</sup>	6	(CA)18	242	60	11	0.534	0.777
FH102 <sup>1</sup>	7	(CT)11(CA)14	179	60	5	0.544	0.557
LA5 <sup>3</sup>	8	(CA)13	130–154	52	6	0.576	0.566
Lat25 <sup>1</sup>	9	(CCAT)15	298–318	52	7	0.712	0.815
Mean					7.556	0.648	0.736
s.d.					2.744	0.087	0.113

<sup>1</sup> (Comstock, Wasser, and Ostrander 2000)

<sup>2</sup> (Archie, Moss, and Alberts 2003)

<sup>3</sup> (Eggert et al. 2000)

<sup>4</sup> Number of alleles per locus, observed heterozygosity (He) and expected heterozygosity (He)

**Supplementary table 2.** A list of individuals carrying each of the 28 distinct mitochondrial DNA haplotypes.

Haplotype	N	Genbank Accession Numbers	Individual labels
Hap_1	1	MF062095	1KF05A
Hap_2	5	MF062096	SKNP08A TEP01A SKNP11A SKNP12A TEP02A
Hap_3	4	MF062097	BZ08A BZ16A BZ17A MAP06A
Hap_4	1	MF062098	NKNP19A
Hap_5	2	MF062099	1PT01A 2MR07B
Hap_6	1	MF062100	MAP03A
Hap_7	1	MF062101	1HW01A
Hap_8	4	MF062102	BZ12A BZ13A NKNP02A SKNP07A
Hap_9	1	MF062103	1HW02A
Hap_10	1	MF062104	BZ05A
Hap_11	1	MF062105	BZ19A
Hap_12	3	MF062106	BMAP02A BZ18A SKNP06A
Hap_13	1	MF062107	BZIM02A
Hap_14	18	MF062108	BMAP01A BMAP03A BZ01A BZ04A BZ06A BZ07A BZ10A BZ11A BZ14A BZ15A BZ20A BZ22A NKNP16A NKNP20A NKNP22A NKNP23A SKNP02A SKNP10A
Hap_15	1	MF062109	1KF07A

Hap_16	1	MF062110	1MR13B
Hap_17	3	MF062111	1CP01A 2SS06A GR03A
Hap_18	5	MF062112	1SN13B 2SN05A GR01A GR02A GR05A
Hap_19	4	MF062113	1CH12A 1CP05A 1MR05A 2CH03A
Hap_20	1	MF062114	2SR05A
Hap_21	61	MF062115	1CH02A 1CH07A 1CH13B 1CP04A 1CP06A 1KF01A 1KF08B 1LV02B 1MR01A 1MR02B 1MR03B 1MR04A 1MR07B 1MR08A 1MR10A 1MR11A 1SN03A 1SN11A 1SR01A 1SR03A 1SR10A 2CP02B 2CP04A 2CP05A 2KA01A 2KA03A 2KA04B 2KA05A 2MR01A 2MR04A 2MR06A 2MR08A 2MR10A 2MR12A 2MR13A 2LY01A 2LY03B 2LY05A 2LY08A 2SN01A 2SN03B 2SN07A 2SN11A 2SR01A 2SR02B 2SR07A 2SR08B 2SR09A 2SR10A 2SR11A 2SS03B 2SS04A 2SS05A 2SS07B 2SS09A 2SS10B 2SS11A LMAP01A MAP02A NKNP01A NKNP10A
Hap_22	20	MF062116	1LV01A 1MR06A 1MR14A 1SN01B 1SN05A 1SN09A 1SN10B 1SN12A 2CH01A 2CH02A 2CP03A 2KA02B 2MR11B 2SN02A GR04A MAP01A NKNP05A NKNP06A NKNP09A NKNP11A
Hap_23	1	MF062117	2SR03A
Hap_24	1	AJ428946.1	
Hap_25	2	EF588275.2 DQ316068.1	
Hap_26	1	EU153451.1	
Hap_27	1	DQ188829.2	
Hap_28	1	JF912200	

N = number of individuals carrying that haplotype. Sampling locality abbreviations: KF = collected from Kafue National Park, Zambia; SKNP and NKNP = southern and northern Kruger National Park, South Africa, respectively; TEP and BMAP = Tembe Elephant Reserve and Maputo Elephant Reserve, Mozambique; BZ = the border of Botswana and Zimbabwe; MAP, LMAP and BZIM = Mapungubwe National Park and along the Limpopo river that connects Mapungubwe and Kruger National Park, South Africa; PT = Pandamatenga, Botswana; MR = Moremi Game Reserve, Botswana; HW = Hwange National Park, Zimbabwe; CP = Caprivi region, Namibia; SS and SN = south and north of Savuti Game reserve, Botswana, respectively; GR = Gonarezhou National Park, Zimbabwe; CH = Chobe National Park, Botswana; SR = Seronga, Botswana; LV = Livingstone, Zambia; LY= Linyanti River, Botswana. Labels for Haplotype 24-28 refer to Genbank Accession numbers of outgroup taxa.

**Supplementary table 3.** Number of elephants and haplotypes of elephants found for each of the mtDNA haplotype clusters identified by Geneland. Number of individuals that carry a haplotype are listed in brackets after the haplotype name.

Cluster Name	Number of individuals	mtDNA Haplotypes
mtDNA Cluster 1	10	Hap_2(5); Hap_3(4); Hap_4(1)
mtDNA Cluster 2	97	Hap_15(1); Hap_16(1); Hap_17(3); Hap_18(5); Hap_19(4); Hap_20(1); Hap_21(61); Hap_22(20); Hap_23(1)
mtDNA Cluster 3	33	Hap_5(2); Hap_6(1); Hap_7(1); Hap_8(4); Hap_9(1); Hap_10(1); Hap_11(1); Hap_12(3); Hap_13(1); Hap_14(18)
mtDNA Cluster4	1	Hap_1(1)

**Supplementary table 4.** Sample size and the mean ( $\pm$  standard error) number of alleles, number of effective alleles, observed heterozygosity, and expected heterozygosity for the different sampling regions in the study area.

	N	Na	Ne	Ho	He
CNP	6	4.000 ( $\pm 0.408$ )	3.124 ( $\pm 0.374$ )	0.602 ( $\pm 0.085$ )	0.644 ( $\pm 0.039$ )
HNP	4	3.778 ( $\pm 0.278$ )	3.091 ( $\pm 0.312$ )	0.741 ( $\pm 0.084$ )	0.651 ( $\pm 0.033$ )
LNP	2	2.000 ( $\pm 0.289$ )	1.889 ( $\pm 0.242$ )	0.389 ( $\pm 0.139$ )	0.375 ( $\pm 0.095$ )
BZB	13	6.333 ( $\pm 0.624$ )	4.243 ( $\pm 0.446$ )	0.767 ( $\pm 0.052$ )	0.740 ( $\pm 0.029$ )
GNP	5	4.444 ( $\pm 0.294$ )	3.517 ( $\pm 0.306$ )	0.661 ( $\pm 0.073$ )	0.694 ( $\pm 0.033$ )
MNP	6	3.444 ( $\pm 0.530$ )	2.439 ( $\pm 0.354$ )	0.504 ( $\pm 0.101$ )	0.493 ( $\pm 0.090$ )
KNP	33	6.444 ( $\pm 0.784$ )	3.956 ( $\pm 0.453$ )	0.626 ( $\pm 0.036$ )	0.712 ( $\pm 0.040$ )

N = sample size, Na = mean number of alleles, Ne number of effective alleles, Ho = observed heterozygosity, He = expected Heterozygosity. Chobe National Park (CNP) = CH, MR, CP, SS, SN, LY; Hwange National Park (HNP) = HW, PT; Livingstone National Park (LNP) = LV; Botswana-Zimbabwe border (BZB) = BZ; Gonarezhou National Park (GNP) = GR, Mapungubwe National Park (MP) = MAP, LMAP, BZIM; Kruger National Park (KR) = NKNP, SKNP

**Supplementary table 5.** Analysis of molecular variance (AMOVA) of seven nDNA regions showed low differentiation between nDNA regions (Fst), with most of the variation attributed to differences within regions.

Source of variation	df	Sum of squares	Estimated variance	Percentage of variation
Among clusters	6	29.457	0.094	3
Within clusters	131	444.456	3.370	97
Total	137	470.913	3.461	100
Fixation index (Fst):	0.027			

**Supplementary Table 6.** Genetic diversity indices for all individuals combined (All) and only individuals found in Kruger National Park (Kruger)

<u>Locus</u>	<u>Number of alleles</u>		<u>Expected heterozygosity</u>	
	All	Kruger	All	Kruger
1	13	11	0.86140	0.84997
2	7	6	0.76947	0.76215
3	6	6	0.77907	0.78312
4	9	7	0.83819	0.82073
5	5	5	0.66143	0.70933
6	11	9	0.77676	0.74576
7	5	3	0.55784	0.51329
8	6	5	0.56604	0.52429
9	7	6	0.81535	0.80995
Mean	7.667	6.444	0.73617	0.72429
s.d.	2.784	2.351	0.11362	0.12376

**Supplementary table 7.** There was no evidence for a recent reduction in effective population size assuming a stepwise mutation model (SMM) or a two-phase model (TPM) for Sign and Wilcoxon test. Prob( $H > H_e$ ) is the probability that the heterozygosity ( $H$ ) is larger than the average ( $H_e$ ) under the null hypothesis, if Prob( $H > H_e$ ) is lower than 0.05, the null hypothesis (mutation drift equilibrium) is rejected in favor of the hypothesis of a recent genetic bottleneck (Cornuet and Luikart 1996).

Test	Model	Prob( $H > H_e$ )
Sign Test	SMM	0.536414
	TPM	0.525722
Wilcoxon Test (1 tailed)	SMM	0.714844
	TPM	0.455078
Wilcoxon Test (2 tailed)	SSM	0.652344
	TPM	0.910156

**Supplementary Table 8.** Sample collection and import was sanctioned the Department of Wildlife and National Parks (Botswana), South African National Parks (SANParks) and the Department of Agriculture, Forestry and Fisheries (DAFF), South Africa and by the Zambian Wildlife Authority. In accordance with the Animal Diseases Act, 1984 (Act 35 of 1984) DAFF permits allowed for the import of elephant dung samples from Botswana, Mozambique, Namibia and Zambia, and a SANParks removal permit allowed the removal of samples from Kruger National Park. Permission was granted to collect samples in Kruger National Park in compliance with section 4(1) of the National Environmental Management: Protected Areas Act 57 of 2003. Original permits are available upon request.

<b>Sanctioning Authority</b>	<b>Permit Description</b>	<b>Permit No:</b>
<b>South African National Parks</b>	Permit to collect natural resources material in the Kruger National Park	SK071
<b>Department of Agriculture, Forestry and Fisheries, South Africa</b>	Veterinary import permit for preserved animal material. Botswana to South Africa. Port of entry Groblersbrug.	13/1/1/30/0-2014/05/001571 and 13/1/1/28/2/10/2-1739
<b>Department of Agriculture, Forestry and Fisheries, South Africa</b>	Veterinary import permit for preserved animal material. Zambia via Zimbabwe to South Africa. Port of entry Beit Bridge.	13/1/1/28/29/8-1742
<b>Department of Agriculture, Forestry and Fisheries, South Africa</b>	Veterinary import permit for preserved animal material. Mozambique to South Africa. Port of entry Lebombo.	13/1/1/28/29/8-1741
<b>Department of Agriculture, Forestry and Fisheries, South Africa</b>	Veterinary import permit for preserved animal material. Namibia to South Africa. Port of entry Nakop/Vioolsdrift.	13/1/1/28/2/10/3-1740
<b>United States Department of Agriculture, United States of America</b>	United States veterinary permit for the importation and transportation of controlled materials and organisms and vectors – Elephant fecal samples	128049 Research



**References:**

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- Eggert LS, Ramakrishnan U, Mundy NI, Woodruff DS (2000) Polymorphic microsatellite DNA markers in the African elephant (*Loxondonta africana*) and their use in the Asian elephant (*Elephas maximus*). Mol. Ecol. 9:2222-2224
- Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. J. Hered. 89, 238–247