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RESEARCH

Reconstructing ancient Southern African mitochondrial genomes at Faraoskop

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Abstract

Twelve human skeletons, approximately 2000 years old, were recovered from the Faraoskop archaeological site in the Western Cape Province, South Africa (Manhire 1993). Several of the skeletons were well enough preserved to determine the osteological profiles (sex, age and stature etc.). Additionally, paleopathological and traumatic changes were observed on some of these skeletal remains. Given suggested context that these human remains were drawn from a single mortuary event, this paper investigates the possibility of familial relationships between the individuals by establishing maternal profiles from mitochondrial DNA. The mitochondrial DNA analysis resulted in the identification of four full genomes from the Faraoskop (FK) individuals and the two Khoesan pastoralist individuals chosen as reference samples for the analysis. Three other FK individuals provided partial genomes which could be assigned to incomplete haplotypes. Five individuals could not be sequenced due to poor DNA preservation. Molecular sex could be confirmed for five FK and two reference individuals, adding to the sex assessment from osteological data. All but one of the mitochondrial haplotypes were L0d1 or L0d2 which is consistent with mtDNA from living Khoesan populations in southern Africa. One individual (FK1) was L0f1, a haplotype which is not present southern African Khoesan, but is currently centred in Uganda and Tanzania. It is occasionally found amongst southern African Bantu speakers which suggests that the presence of L0f1 is a remnant of an earlier distribution which is now lost. The three L0 mitochondrial haplotypes from the six Faraoskop individuals (L0d1, L0d2, and L0f) suggest a diversity of maternal lineages compatible with the diversity of Khoesan groups but given the simultaneity of the burial, it is tempting to suggest that those with similar maternal haplotypes were closely related.

Keywords Mitochondrial DNA · Southern Africa · Later Stone Age · Khoesan

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Introduction

South Africa has an extensive hominid fossil history, which goes back more than two million years. Contemporary indigenous populations from Southern Africa display a high genetic diversity, and harbour early divergent lineages (Wang et al. 2020; Schlebusch et al. 2020; Al-Hindi et al. 2022). However, there is little direct archaeological information that can demonstrate the development of this diversity in the deep time scale of human occupation. Analysis of human skeletal remains from the early and middle Holocene have shown that skeletal and dental morphology have changed, (Stynder et al. 2007; Irish et al 2014; Cameron et al 2021), but very little is known about the genetic history that accompanied these changes. Only in relatively recent time has ancient DNA (aDNA) become available for individuals found in archaeological contexts from Africa. The difficult preservation conditions and the overall paucity of ancient human remains have been hampering the retrieval of reliable aDNA in the early years (Vicente and Schlebusch 2020). Mitochondrial DNA (mtDNA) is a valuable resource for ancient DNA studies. Before the advent of automated genomic analysis (Reich 2019), mtDNA was seen as the golden standard of genetic research because both the abundance of mitochondria in the cell and the ease of retrieving the relatively short mtDNA sequences in the laboratory (Pakendorf and Stoneking 2005). The analysis of mitochondrial genomes remains valuable especially in studies where maternal gene flow between small groups is of interest (Merheb et al 2019, Diallo et al. 2022). In one of the first African aDNA studies, Morris et al. (2014) described the first complete ancient mitochondrial genome from a pre-pastoral inhabitant of St Helena Bay in the Western Cape Province of South Africa. This ancient mitochondrial genome was identified as a new L0d2c lineage, which is closely related to contemporary indigenous San speakers (specifically the Jul'hoan speakers of the Kx'a language family (Barbieri et al. 2013). Further mtDNA genomes from other ancient South African skeletons have now been published including one from the Faraoskop site itself (Skoglund et al. 2017), a child from the pastoralist site of Kasteelberg (Skoglund et al. 2017) and three Later Stone Age foragers from coastal KwaZulu-Natal (Schlebusch et al. 2017). Their haplogroups belong to major branches of the mtDNA phylogeny which are still found predominantly in southern Africa: these are L0d1 and L0d2, frequent in contemporary San hunter gatherers and in neighboring Khoe-speaking pastoralist and hunter gatherer groups, and L3e, an ubiquitous sub-Saharan lineage most frequently found in Bantu speaking groups and in Central Africa (Soares et al. 2012).

We present here seven additional reconstructed ancient African mitochondrial genomes from Southern Africa. The aim of this study is to add molecular data to the bioanthropology of the unique deposition site of Faraoskop. The objectives are threefold: 1) to add to the general body of published ancient DNA results from southern Africa; 2) to provide complementary genetic sex determination for individuals who have been identified as a specific sex through morphology, and to include other individuals who are too poorly preserved for osteological observation; and 3) to explore the genetic relationships for a set of individuals who are almost certainly from one community drawn from a precise moment in time in the Later Stone Age of South Africa. The genetic profiles from the maternally inherited mtDNA genomes will give a further contextualization of the relationship between these individuals and the surrounding genetic landscape of people living today in different regions of sub-Saharan Africa.

The human skeletons described in this study were recovered from a rock shelter on the Faraoskop Hill in the Western Cape Province of South Africa. The site lies near the town of Graafwater at 32°07′31″S and 18°36′52″E. The rock shelter faces west, overlooking the coastal plain and is protected by a rocky outcrop. The rock shelter is approximately 5 m wide and 8 m high and is obscured by a wall constructed by the local landowner in 1984. Previously, the Faraoskop skeletons, which form the basis of the present investigation, were studied to determine diet and dental caries (Sealy et al. 1992). The discovery of marine shells in the Faraoskop deposits, which is about 35 km from the coast, is evidence of contact with the ocean; however, the skeletons have $\delta^{13}C$ values which suggest a low intake of marine food for these individuals (Sealy et al. 1992; Gray et al. (n.d.) this volume). The archaeology of the Faraoskop shelter indicates that the site was occupied by Later Stone Age (LSA) foragers rather than pastoralists (Manhire 1993).

The excavated skeletons along with those previously discovered by the local landowner represent an exceptional depositional context for the region: a group of individuals buried together as the result of what may have been a single death event. Other multiple burials from the LSA in southern Africa either cannot be confirmed as being contemporaneous or involve only two or at most four individuals (Pfeiffer et al. 2020). The case for contemporaneity at Faraoskop is based on three lines of evidence: radiocarbon dating; evidence of violence at death; and what appears to be a single burial horizon.

Six of the Faraoskop individuals, including three skeletons from the farmer's finds and three skeletons excavated by the Archaeology Department at UCT in 1988, were radiocarbon dated soon after excavation and in 2014 five more skeletons were dated. Overall, the dates suggest that

the burial event happened shortly before or around the turn of the first millennium CE.

Parkington & Dlamini (2015) and Dlamini and Morris (2024) have noted that up to six (50%) of the individual skeletons showed perimortem cranial injuries. It is theoretically possible that half of the individuals died from separate violent interpersonal trauma over separate years, but this is extremely unlikely. The simplest explanation is that the burials at Faraoskop consist of several people who were attacked and suffered cranial trauma in one violent event.

Only five of the skeletons were excavated under archaeological control (Manhire 1993) and much of the site had been disturbed through the actions of the local landowner, through continued prehistoric occupation of the site after the burial events and by animal bioturbation. Despite that, the re-examination of the original descriptive field notes and photographic record of the excavation make it clear that none of the excavated five burials can be separated from the other four. The individuals were in articulated alignment but crammed together in a confined space indicating a single burial. Although there were no records of the original placement of the bones removed by the landowner, the presence of at least one cranial element from the farmer's collection matching one of the excavated skeletons strongly suggests that the unexcavated remains were in a context continuous with the excavated sequence. Taken together, the evidence from dating, violent death and burial association is overwhelmingly in support of a single burial event.

Manhire (1993) provided an anthropometric analysis which identified a minimum number of individuals of ten adults and two sub-adults, based on the morphological analysis of Alder (1988). During the cleaning and reconstruction of the samples for this study, it was noted that UCT 390 consisted of a cranium and post-cranial bones of two different individuals. The cranium of UCT 390 was subsequently assigned to the post-cranial bones of UCT 391 which had been accessioned without a cranium.

Samples from two additional individuals who do not belong to the Faraoskop site have been included as references for genetic analysis. These are from early pastoralist sites: UCT 437 from Kasteelberg (Smith 2006) and UCT 582 from Voelvlei 1 (Morris et al. 2005). These two individuals form a Later Stone Age outgroup of Khoe pastoralists for comparison. Table 1 provides basic demographic data for all fourteen skeletons described.

Methodology

We present here genetic results obtained as part of a screening for preservation of authentic ancient DNA in a collection of LSA South African human skeletal remains. Beyond assessment of authenticity and quality of the data, this

Table 1 Summary of the age, morphological sex and bone sample type for the individuals in this study

Skeletal ID	UCT ID	Age	Sex (morphology)	Sample Type
FK 1	UCT 385	adult	Undetermined	Right femur
FK 2	UCT 386	40 – 50 yrs	Male	Right femur RUM3/premolar
FK 3	UCT387/UCT 392	30—40 yrs	Male	RUM3 Right pelvis
FK 4	UCT 388	6 −8 yrs	Undetermined/ juvenile	Right Radius
FK 5	UCT389/UCT 397	35—45 yrs	Undetermined	LLM2 Left Humerus
FK 6	UCT 390 (cranium) /UCT 391	20—25 yrs	Female	RUM3 Right femur
FK 7	UCT 390 (post-cranial)	30 – 40 yrs	Undetermined	Tibia
FK 8	UCT 393	2—3 yrs	Undetermined/ juvenile	Foot Phalanx
FK 9	UCT 394	45 – 55 yrs	Male	Right femur Tooth
FK 10	UCT 395	25–30 yrs	Female	Left femur
FK 11	UCT 396	35 – 45 yrs	Female	Left femur
FK 12	UCT 398	40 – 50 yrs	Undetermined	Right humerus
Kasteelberg	UCT 437	±4 yrs	Undetermined/ juvenile	Right femur RLM1
Voelvlei 1	UCT 582	30–40 yrs	Female	Right Femur Tooth

approach allows for sex identification and reconstruction of the mitochondrial genomes of the skeletal remains, supplementing the archaeological and osteological evaluations. Sufficient endogenous DNA content for cost-efficient deep shotgun sequencing was observed for two of the samples (Table 2), FK 2 (UCT 386) of the Faraoskop series, and the comparative individual UCT 437, and subsequent whole genome analysis on these was completed as part of a separate study (Skoglund et al 2017). Considering the difficulty of obtaining any authentic aDNA from the African continent, especially without skeletal elements that have been shown to have exceptional nuclear DNA preservation, i.e. the petrous bone and ossicles (Pinhasi et al. 2015; Sirak et al. 2020), targeted enrichment approaches, such as for the complete mtDNA genome, provide a good middle-ground to obtain data for genetic genealogical analysis. Furthermore, the mtDNA sequences for southern African populations are well understood and comparisons with data from living populations can be fruitful.

In order to reduce the risk of additional contamination of the skeletal remains, DNA extraction was performed in a dedicated ancient DNA facility at the University of Tübingen, Germany. A total of 17 samples from Faraoskop were collected for this study, plus two samples each from the two pastoralist reference individuals. The preferred sample type, a section from the distal end of the femur, was removed. If the femur was unavailable, a cortical sample was taken from another long bone. Additionally, a tooth sample was collected for all individuals where available.

To determine whether DNA was preserved in these remains, several sections of bone or teeth from the various individuals were examined. Bone powder was obtained from 14 of the samples and dental powder from the internal root canal of 7 tooth samples. DNA was extracted using an

Table 2 Results of the shotgun sequencing

Skeletal ID	UCT ID	# of Raw Reads prior Clip & Merge	# reads after Clip&Merge prior mapping	# mapped quality-filtered reads prior RMDup	# mapped quality-filtered reads after RMDup	Endogenous DNA (%)	G-to-A 1st Base 3'-end	C-to-T 1st Base 5'-end	average fragment length
FK 1	UCT385	1,742,232	811,667	3,764	3,749	0.465	0.3805	0.3676	40.13
FK 2	UCT386 femur	2,662,186	1,370,551	3	3	0	0	0	30.33
FK 2	UCT386 tooth	3,242,274	1,503,374	400,692	399,943	26.963	0.3651	0.3845	42.55
FK 3	UCT387	3,290,192	1,576,669	75	75	0.005	0.2143	0.2857	34.65
FK 3	UCT392	112	58	0	0	n/a	n/a	n/a	n/a
FK 4	UCT388	2,695,144	1,330,894	82	82	0.007	0.2941	0.1875	38.82
FK 5	UCT389	1,886,246	889,071	138	138	0.016	0.3889	0.2174	37.64
FK 5	UCT397	1,423,282	667,597	31	31	0.005	0	0	38.9
FK 6	UCT390 tooth	3,659,870	1,770,228	5,337	5,325	0.319	0.4019	0.4266	40.89
FK 6	UCT391	1,680,608	825,825	1,111	1,110	0.142	0.3693	0.4157	46.69
FK 7	UCT390 tibia	1,996,588	707,052	1,231	1,202	0.175	0.3101	0.3892	37.96
FK 8	UCT393	1,506,250	785,688	509	506	0.078	0.2047	0.3226	57.3
FK 9	UCT394 femur	4,202,558	1,912,983	30,090	29,918	1.579	0.2787	0.2869	42.82
FK 9	UCT394 tooth	18,358,138	7,881,312	5,687	5,655	0.072	0.2603	0.299	39.11
FK 10	UCT395	1,181,310	552,591	2,364	2,351	0.43	0.3402	0.3208	42.3
FK 11	UCT396	1,978,194	914,968	17,895	17,776	1.963	0.3251	0.3196	41.06
FK 12	UCT398	2,538,046	1,025,293	66	66	0.007	0	0	36.48
Kasteelberg	UCT437 femur	2,706,796	1,214,084	61,421	61,179	5.073	0.2485	0.2567	44.33
Kasteelberg	UCT437 tooth	1,553,022	745,009	10,574	10,512	1.499	0.3114	0.3367	51.9
Voelvlei 1	UCT582 femur	1,230,680	583,576	8,368	8,322	1.448	0.2472	0.2673	49.52
Voelvlei 1	UCT582 tooth	2,166	954	29	29	3.04	0.3333	0.4615	40.72

established protocol (Dabeny et al. 2013) with modifications as described in Andrades-Valtueña et al. 2017. The extracted DNA was converted to double-indexed libraries (Meyer and Kircher 2010; Kircher et al. 2012) in preparation for sequencing. Negative controls were included during extraction and library preparation.

Subsets of the DNA libraries were prepared for shotgun sequencing on the Illumina Platform. Shotgun sequencing allows one to make inferences about the preservation of the sample, the endogenous DNA present and the sex of individuals. The libraries were also enriched for mitochondrial DNA, using present-day human mitochondrial amplification product as the probe (Maricic et al. 2010). Paired-end sequencing was carried out on the Illumina HiSeq2500 platform for $2\times101+2\times8$ cycles.

After demultiplexing, resulting sequencing reads were processed using EAGER, a computational pipeline developed for aDNA (Peltzer et al. 2016) that merges paired-end reads (default parameters) and maps reads utilizing BWA (v0.6.1) (Li and Durbin 2009) against a user-specified reference genome. The shotgun data was mapped against the UCSC genome browser's human genome reference GRCh37/hg19, resulting in 3 to 399,943 quality-filtered unique reads per sample. We failed to retrieve the expected amount of raw sequencing reads for the samples UCT392 and UCT582 tooth, likely due to a pipetting error during generation of the DNA libraries (Table 2).

The molecular genetic sex determination was performed on the shotgun sequence data by comparing the number of reads mapping to the X chromosomes and to the Y chromosome as well as the autosomes (Skoglund et al. 2013; Mittnik et al. 2016).

The data from mitochondrial capture were mapped to the revised Cambridge reference sequence (rCRS) (Andrews et al. 1999) using the circular mapper implemented in EAGER.

To confirm the authenticity of the human ancient DNA we used mapdamage 2.0 (Jónsson et al. 2013) to estimate deamination pattern typical of ancient DNA, by observing typical patterns of C to T base misincorporation caused by deamination of cytosine to uracil towards 5' read ends, and G to A misincorporations towards 3' read ends (Sawyer et al. 2012). The damage patterns of our samples are illustrated in Fig. 1. We observe in all libraries for which enough reads were sequenced expected frequencies of misincorporation of at least 0.15 at either end except in UCT397 and UCT398. Fragment length is also indicative of sample age and is typically between 30 and 100 bp in ancient DNA samples. The DNA fragments presented here are between around 30 bp – 70 bp (Tables 2 and 3), which confirms the authenticity of the ancient sample (Jónsson et al. 2013). The software Schmutzi was used to estimate modern contamination in the mtDNA-capture data and confirm the integrity of the endogenous aDNA, as well as generate consensus sequences (Renaud et al 2015).

The mtDNA sequences were aligned together with a reference dataset of 710 mtDNA sequences (Barbieri et al. 2013, 2014b; Behar et al. 2008; Brucato et al. 2018; Chan et al. 2015; Gonder et al. 2007; Oliveira et al. 2018; Rito et al. 2013; Schlebusch et al. 2017; Uren et al. 2016), from the three haplogroups retrieved L0f, L0d1 and L0d2, using the software MUSCLE (https://www.ebi.ac.uk/Tools/msa/muscle/). The alignment was visually inspected and the two polyC sections were trimmed (as prone to sequencing and alignment errors). Haplogroups were assigned using Haplogrep3 v.3.2.1 (Schönherr et al. 2023) with the rCRS, the Phylotree 17.2 phylogeny and default settings. Comparative analyses were conducted separately for the three haplogroup using R and packages ape (Paradis et al. 2004), pegas (Paradis 2010) and ggplot2 (Wickham 2016) for network visualization. Networks were built using a selection of the sequences closer to our newly sequenced data. Pairwise genetic distances between sequences (Kimura's 2-parameters distance) are calculated with the function dist.dna.

Results

A total of 14 newly sequenced individuals were included in this study; 12 from the Faraoskop site, and UCT 437 and UCT 582. Kasteelberg (UCT 437) and FK2 (UCT 386) have been published previously (Skoglund et al. 2017), but the data presented here are a re-analysis using the same methodology as the other individuals.

Sex determination

The Faraoskop skeletal assemblage was previously examined and the morphological sex assessment was performed by evaluating defined metric criteria of the pelvic bone and the cranium (Ubelaker 1978). Morphological methods have proved useful in the assessment of sex in adult skeletons, although they are less reliable for juveniles and incomplete skeletons. The molecular determination of sex often relies on sex specific marker loci such as the amelogenin gene (e.g. Gibbon et al. 2009), focusing on the dissimilarity between the X-linked amelogenin gene and the amelogenin on the Y-chromosome, but prone to error due to allelic dropout and contamination (Kim et al. 2013). In this paper we used shotgun sequencing data to reliably identify chromosomal sex simply by considering R_y, the ratio of the sequences aligning to the X and the Y chromosomes (Skoglund et al. 2013), or R_x, the ratio of reads aligning to the X chromosome and the autosomes (Mittnik et al. 2016). The molecular genetic sex determination was then compared to the morphological sex identification. The reliability of genetic sex determination is

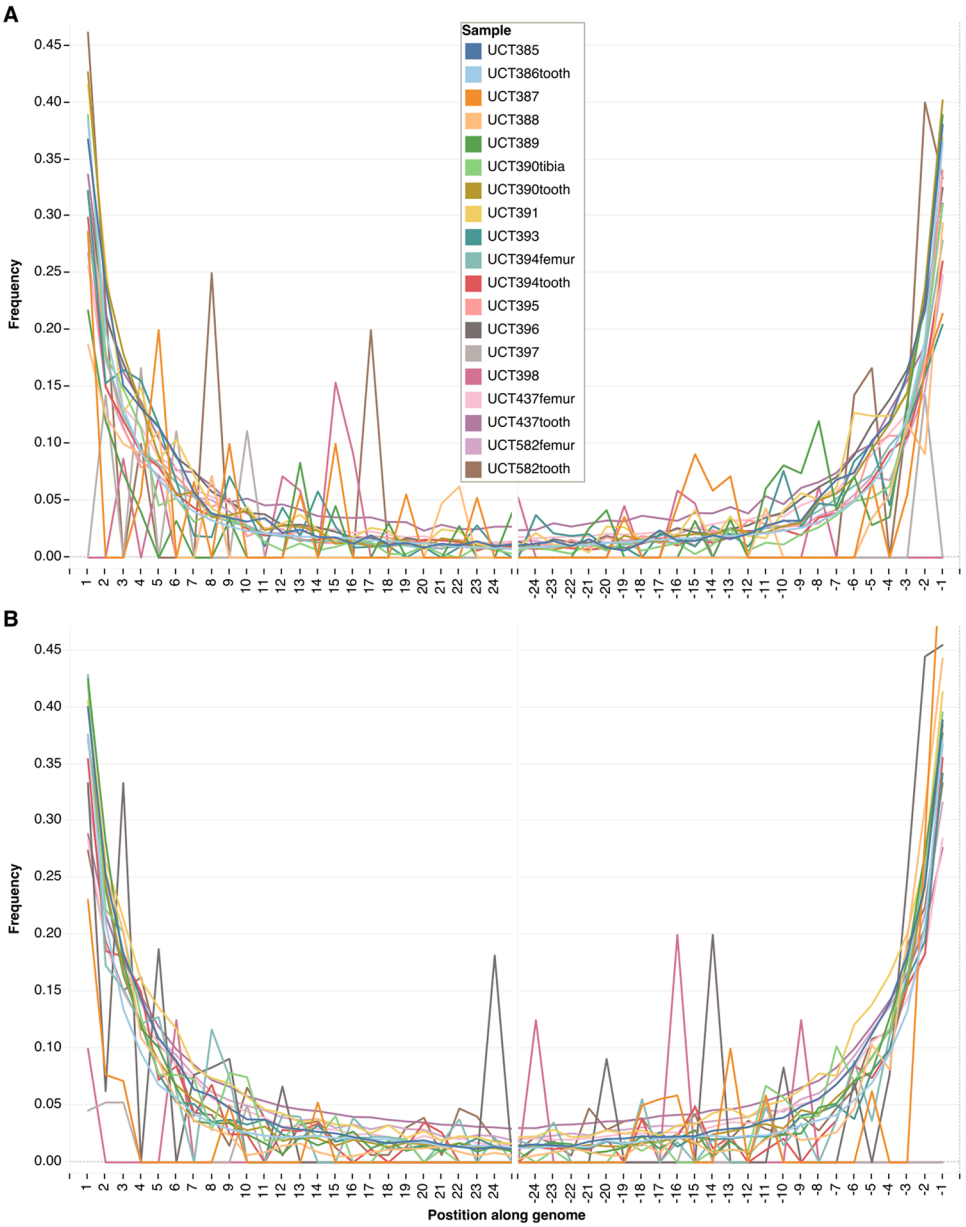

Fig. 1 Misincorporation plots for all samples with over 10 quality-filtered mapped reads. Damage pattern shown for shotgun data (**A**) and mtDNA capture data (**B**). Left-hand side of plots shows C-to-T misincoporation frequency from the 5'-end of the read and right-hand side of the plots shows G-to-A misincorporation frequency from the 3'-end of the reads

dependent on sequence coverage in the analysis and uncertainty in the assignment is reflected when the ranges of R_y and R_x do not fully fall within the ranges consistent with the expectation for XX or XY (Table 3).

Table 2 summarises the genetic data derived from shotgun sequencing of the skeletal remains from Faraoskop and Table 3 the sex identification derived from this data. Of the 14 individuals under consideration, seven individuals could not be sexed morphologically, four because the skeletons were too incomplete, and three because the individuals were immature at death and the sexually dimorphic features had not yet developed.

Molecular sex determinations could confidently be made for seven of the individuals (FK1, FK2, FK6, FK9, FK11, UCT437, UCT582), while the estimation for four individuals held uncertainty (list FK 3,7,8,10 numbers) and was not possible at all for the remaining three (FK4, FK5 and FK 12; Table 3). FK8 had fewer than 1,000 mapping reads and the sex determination as XX is therefore not confident and should be considered to be uncertain. We caution that the sex determination using R_x is uncertain for samples with fewer than 1,000 reads mapping to the human genome, and for fewer than 10,000 reads using R_y. Where both methods provided a sex estimation, they agreed in all but one case (FK1), where we accept the XY assignment with the R_x method due to the number of mapping reads falling below 10,000.

Table 3 Sex determination results

Sample	Morphological assignment	Unique reads mapping to X	Unique reads mapping to Y	Skoglund et al 2013 method (R_y)			Mittnik et al 2016 method (R_x)		
				R_y	95% CI	Assignment	R_x	95% CI	Assignment
FK1 UCT385	Undetermined	86	3	0.0337	−0.0038 to 0.0712	consistent with XX but not XY (< 10,000 reads)	0.4445	0.4147 to 0.4744	XY
FK2 (femur) UCT386	Male	0	0	Not assessable			Not assessable		
FK2 (tooth) UCT386	Male	9706	917	0.0863	0.081–0.0917	XY	0.4748	0.4526 to 0.4970	XY
FK3 UCT387	Male	2	0	Not assessable			0.6227	0.4864 to 0.7590	consistent with XY but not XX (< 1,000 reads)
FK4 UCT388	Undetermined/ juvenile	4	1	0.1789	−0.1506–0.5506	not assigned	Not assessable		
FK5 (tooth) UCT389	Undetermined	8	2	0.1265	−0.0479–0.4479	not assigned	Not assessable		
FK5 (humerus) UCT397	Undetermined	1	0	Not assessable			Not assessable		
FK6 (tooth) UCT390	Female	258	2	0.0054	−0.0029–0.0183	consistent with XX but not XY (< 10,000 reads)	0.9899	0.9253 to 1.0545	XX
FK6 UCT391	Female	61	0	Not assessable			1.1084	1.0155 to 1.2012	XX
FK7 (tibia) UCT390	Undetermined	33	1	0.029	−0.0274–0.0862	not assigned	0.5495	0.4946 to 0.6045	consistent with XY but not XX
FK8 UCT393	Undetermined/ juvenile	27	0	Not assessable			1.1134	1.0245 to 1.2022	XX (< 1,000 reads)
FK9 (femur) UCT394	Male	746	86	0.0106	0.0827–0.1241	XY	0.4835	0.4574 to 0.5096	XY
FK9 (tooth) UCT394	Male	147	12	0.0209	0.0344–0.1165	consistent with XY but not XX (< 10,000 reads)	0.5115	0.4878 to 0.5351	XY
FK10 UCT395	Female	99	1	0.0099	−0.0095–0.0295	consistent with XX but not XY (< 10,000 reads)	0.8526	0.7855 to 0.9196	consistent with XX but not XY
FK11 UCT396	Female	832	5	0.0027	0.0008–0.0112	XX	0.9268	0.8818 to 0.9719	XX
FK12 UCT398	Undetermined	4	0	Not assessable			Not assessable		
UCT437 (femur)	Undetermined/ juvenile	2817	20	0.0016	0.004–0.0101	XX	0.9095	0.8648 to 0.9543	XX

Table 3 (continued)

Sample	Morphological assignment	Unique reads mapping to X	Unique reads mapping to Y	Skoglund et al 2013 method (R_y)			Mittnik et al 2016 method (R_x)		
				R_y	95% CI	Assignment	R_x	95% CI	Assignment
UCT437 (tooth)	Undetermined/ juvenile	510	2	0.0028	−0.0015–0.0093	XX	1.0098	0.9640 to 1.0555	XX
UCT582 (femur)	Female	393	2	0.0036	−0.0019–0.0121	XX (< 10,000 reads)	0.9392	0.8940 to 0.9845	XX
UCT582 (tooth)	Female	3	0	Not assessable			Not assessable		

Mitochondrial genome analysis

For 4 Faraoskop individuals (FK1, FK2, FK6, and FK8) and UCT 437 and 582 we could reconstruct the full mitochondrial genome with fewer than 4% of bases missing and no evidence of contamination (Table 4). For a further 2 individuals (FK4 and FK5), partial mtDNA genomes with a missing rate of 23 to 33% could be recovered, providing sufficient coverage for haplogroup assignment and low contaminations estimates. FK9, for which we pooled the sequencing data from two skeletal samples, yielded enough coverage for a tentative haplogroup assignment (69% missingness), but not enough to assess contamination. DNA preservation was not sufficient to recover usable genomes for 5 individuals (FK3, FK7, FK10, FK11, FK12; Table 4). Haplogroup assignment showed the presence of haplogroups L0f, L0d1 and L0d2, all lineages of macro haplogroup L0, characteristically present in sub-Saharan African populations (Barbieri et al. 2013). The presence of diagnostic positions (especially outside the hypervariable region) is considered for assignment of downstream sub-haplogroups. Individual FK9 displays mutation 719A associated with haplogroup L0d1 and 9111C associated with haplogroup L0d1b2b1. Individuals FK4 and FK5 are assigned to L0d2 and possibly to sublineage L0d2a1 according to the presence of further downstream mutations: individual FK4 harbours mutation 11854C for L0d2, and 597 T, 8392A, 12234G and 12810G for L0d2a1; individual FK5 harbours mutations 7154G, 11854C, 15766G for L0d2 and 597 T and 12810G for L0d2a1.

Further inspection of the mtDNA genomes aims at finding possible relatedness between the target individuals and other living and ancient individuals included in our comparative dataset.

1) FK6, a female in her early 20 s, FK8, a child of 2–3 years of age, and FK4, a child of 6–7 years of age share the same haplogroup L0d2a1 and are possibly related. FK6 has two additional private mutations at 15,061 and 16,293. FK8 shares the same private mutation at 15,061. This individual has worse coverage and no read covering position 16,293 so we can't say if the two individuals share fully identical haplotypes, but the one shared private mutation is an indication that they might.

A third older male individual, FK5, could not be assigned to the same full haplogroup but shared the L0d2 sequence. The coverage for this individual is much worse, but 2 of the 6 defining mutations for L0d2a1 are there so that is the most likely haplogroup assignment. This individual is not covered at 15061 but is covered at 16293. The latter position does not carry the same private mutation as FK6 (evidence of absence) so we can exclude that these two individuals carry the identical haplotype. We cannot exclude that FK8 and FK5 carry the same haplotype (absence of evidence at both positions), so there remains a distinct possibility that these three individuals share a common female ancestor.

2) FK2, an older male, provided a complete mitochondrial haplotype which has been assigned to the haplogroup L0d1b2b1b, indicating that his maternal ancestor was different from the other analysed individuals.
3) One individual, FK1, a male in his 20 s, has the entirely unrelated haplogroup L0f1, indicating that his maternal ancestor was also different from the other analysed individuals.

The two outgroup individuals, UCT 437 and 582, both aboriginal pastoralists, produced highly resolved genomic results, with the former assigned to a L0d1a1a haplotype, and the latter to a L0d2ala haplotype.

The newly generated mitochondrial genomes were compared with a panel of mitogenomes available for each macro haplogroup. Figure 2 shows the network of relatedness between haplotypes, by displaying the closest link for L0d1 and L0d2, and all the available L0f mitogenomes. The L0f lineage of FK1 is rare, represented only sporadically in South Africa (Behar et al. 2008). It is more frequently found in Bantu-speaking groups in central and eastern Africa

Table 4 Mitochondrial genome sequencing and haplogroup assignment results

Skeletal ID	UCT ID	# of Raw Reads prior Clip & Merge	# reads after Clip & Merge prior mapping	# mapped reads prior RMDup	Mapped Reads after RMDup	Average coverage on mtDNA genome	G-to-A 1st Base 3'-end	C-to-T 1st Base 5'-end	average fragment length	Schmutzi contamination estimate	mt-genome covered by consensus (%)	Haplogrep haplogroup assignment
FK 1	UCT385	4,743,006	2,242,672	202,422	48,425	126.568	0.3885	0.4003	43.25	0.01–0.03	99.73	L0f1
FK 2	UCT386femur	987,116	504,834	1	1	0.002	1	0	31	n/a	n/a	n/a
FK 2	UCT386tooth	5,042,544	2,400,177	102,705	68,150	193.648	0.371	0.3757	47.03	0–0.02	99.99	L0d1b2b1b1
FK 3	UCT387	6,607,228	3,137,396	81	68	0.184	0.5625	0.2308	44.81	n/a	n/a	n/a
FK 3	UCT392	2	1	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
FK 4	UCT388	4,944,110	2,394,978	2,567	1,502	3.750	0.4429	0.4005	41.29	0–0.02	67.42	L0d2a1
FK 5	UCT389	5,060,100	2,364,152	2,757	1,754	4.657	0.3772	0.4248	43.91	0–0.02	77.31	L0d2a1
FK 5	UCT397	3,537,768	1,606,006	650	94	0.346	0	0.0455	60.98	n/a	n/a	n/a
FK 6	UCT390tooth	2,491,612	1,202,452	23,828	15,374	41.949	0.3844	0.3725	45.11	0–0.02	99.68	L0d2a1
FK 6	UCT391	4,924,714	2,409,669	13,487	10,039	30.297	0.4135	0.4058	49.84	0.01–0.03	99.36	L0d2a1
FK 7	UCT390tibia	3,580,520	1,271,763	15,344	409	0.938	0.3956	0.4205	37.91	n/a	n/a	n/a
FK 8	UCT393	6,150,876	3,171,774	5,659	3,685	11.620	0.3419	0.3759	52.1	0–0.02	96.30	L0d2a1
FK 9	UCT394femur	99,704	44,851	238	219	0.562	0.3667	0.4286	42.38	n/a	n/a	n/a
FK 9	UCT394tooth	4,807,850	2,032,770	367	333	0.801	0.3556	0.3544	39.61	n/a	17.99	L0d1
FK 9	UCT394_merged	9,731,396	2,077,621	605	552	1.363	0.36	0.3852	40.71	n/a	31.23	L0d1b2b1
FK 10	UCT395	100,742	46,469	5	4	0.009	0	0	37.75	n/a	n/a	n/a
FK 11	UCT396	14,424	6,699	149	51	0.133	0.4545	0.3333	43.31	n/a	n/a	n/a
FK 12	UCT398	5,478,194	2,214,342	99	39	0.175	0	0.1	71.87	n/a	n/a	n/a
Kasteelberg	UCT437femur	1,860,900	872,482	87,320	49,423	139.977	0.2847	0.2833	46.84	0.01–0.03	99.98	L0d1a1a
Kasteelberg	UCT437tooth	2,932,374	1,411,032	631,945	369,345	1584.721	0.2764	0.2888	71.03	0.01–0.03	100.00	L0d1a1a
Voelvlei 1	UCT582femur	1,127,226	542,935	63,935	34,066	106.147	0.3164	0.3262	51.51	0.01–0.03	99.98	L0d2a1a
Voelvlei 1	UCT582tooth	3,844	1,776	728	681	1.873	0.3333	0.2742	45.46	0–0.02	38.87	L0d2a1a

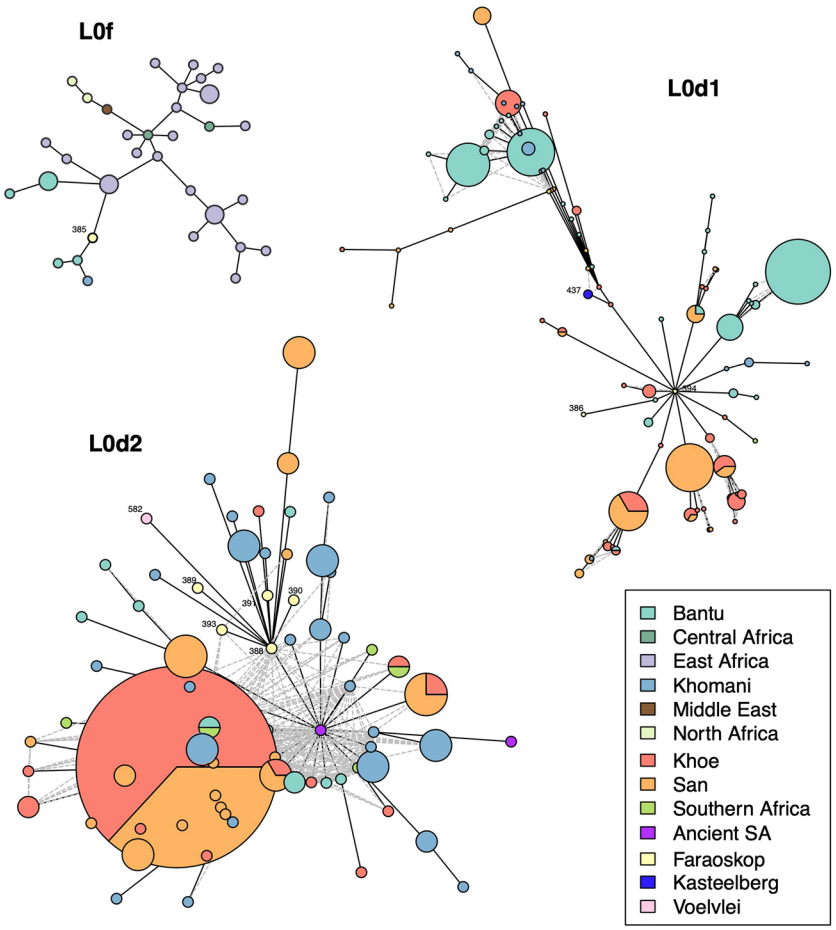

Fig. 2 Network showing phylogenetic connections between the haplotypes retrieved. Circle size is proportional to the number of individuals per haplotype. The newly analyzed data is highlighted with the ID numbers of the individuals. Only the closest sequences to individuals in L0d1 (Kimura's 2-parameters distance < 0.008) and L0d2 (Kimura's 2-parameters distance < 0.005) are considered, to make the networks more readable. Individuals from published literature are associated to broad groups, highlighted by different shades of grey, but in colour online. The groups are based on geographic and/or ethnolinguistic criteria

(Rito et al. 2013). L0d1 is the most represented haplogroup in Southern African Khoesan groups (Barbieri et al. 2013, 2014a). The Kastelbeerg individual UCT437 is located on a distinct branch, together with other individuals from the L0d1a1a sublineage, and loosely related to two Khoe and one San individuals. FK9 is represented as a merge of two low quality sample extractions (femur and tooth) which has resulted in an incomplete mitochondrial genome. It appears at the center of a starlike pattern possibly because of the lack of characteristic private mutations. Individual FK2 is located in this section of the network together with FK9 and other Khoe, San, Khomani and Bantu speakers. The network of relatedness for L0d2, reconstructed around our newly genotyped individuals, is tighter than the network reconstructed for L0d1. On the left of the figure, the L0d2 network is dominated by a single haplotype shared by a large number of Khoe and San individuals. Our Faraoskop individuals are located in a region of the network with a high presence of Khomani individuals, one or two mutations apart from each other. Voelvlei (UCT582) is also connected to this section of the network, yet a few mutations more distant from the Faraoskop individuals.

Discussion

Nearly all research on mitochondrial DNA in Southern Africa has been done making use of the extensive data set of DNA samples from living Bantu speaking agriculturalists, San hunter-gatherers and Khoekhoe pastoralists. Only very recently has mtDNA been extracted from ancient skeletal remains. Morris et al. (2014) published an L0d2c1c haplotype for a forager from St Helena Bay roughly contemporaneous with the Faraoskop individuals. Ancient genetic data from seven individuals from the coastal region of KwaZulu-Natal province in South Africa have also been examined (Schlebusch et al. 2017; Pfeiffer et al. 2019). Of these seven individuals, four are from archaeological contexts indicating prehistoric foragers and are directly comparable to the Faraoskop sample. Three of the KwaZulu-Natal individuals are also similar in time to Faraoskop – between 1900 and 2300 years ago. When UCT 437 and 582, representing a Khoekhoe 'outgroup' in this study, are included, there are in total 7 ancient mitochondrial genomes available for comparison to the Faraoskop genomes (Table 4).

Table 5 Comparison of mtDNA haplogroups for ancient individuals

Skeletal ID and UCT Number	Age and Sex	Mitochondrial Haplotype	Source
L0d1 individuals			
UCT 437 (Kasteelberg)	Juvenile female	L0d1a1a	This study
FK 9—UCT 394	Adult male	L0d1b2b1	This study
FK 2—UCT 386	Older adult male	L0d1b2b1b	This study
L0d2 individuals			
DBN 3062 (Doonside)	Adult female	L0d2	Schlebusch et al. 2017
FK 4—UCT 388	Juvenile male	L0d2a1	This study
FK 5—UCT 389/397	Older adult male	L0d2	This study
FK 6—UCT 390/391	Adult female	L0d2a1	This study
FK 8—UCT 393	Young child female	L0d2a1	This study
KZN Museum 2009/008 (Ballito Bay B)	Adult male	L0d2a1	Schlebusch et al. 2017
UCT 582 (Voelvlei)	Adult female	L0d2a1a	This study
KZN Museum 2009/023 (Champaign Castle)	Adult female	L0d2a1a	Schlebusch et al. 2017
KZN Museum 2009/007 (Ballito Bay A)	Juvenile	L0d2c1	Schlebusch et al. 2017
UCT 606 (St Helena Bay)	Adult male	L0d2c1c	Morris et al. 2014
L0f individuals			
FK 1—UCT 385	Adult male	L0f1	This study

L0d haplotypes are present in 12 of the 13 ancient skeletons in Table 5. The four individuals from KwaZulu-Natal are all L0d2 (Schlebusch et al. 2017). One shares a subdivided lineage of L0d2c1 with the individual from St Helena Bay, and one other share a subdivided lineage of L0d2a1 with UCT 582 and three individuals from Faraoskop. Of the ancient individuals, only UCT 437 shares an L0d1a haplogroup with two of the Faraoskop individuals, but its subdivided haplogroup of L0d1a1a is distinctive.

The L0 mitochondrial branch traces back its origin to the earliest split of the anatomically modern humans, which started to differentiate in further sub-branches around 180,000 years ago (Rito et al. 2013, Chan et al. 2015, 2019, Soares et al. 2016). The geographic location of where the L0 first started to differentiate is speculated to have been in sub-Saharan Africa: either in southern Africa (Henn et al. 2011; Rito et al. 2013; Soares et al. 2016), eastern Africa (Nielsen et al. 2017) or northern Botswana (Chan et al. 2019). The great time depth for the L0 branch is supported by the fact that although only about 11% of modern Africans have this haplotype, the different sub-branches of L0 account for 50% of mtDNA diversity in all living humans (Rito et al. 2013). The split between L0 and the L1-6 clades at around 100,000 years ago is paralleled by the separation of Khoesan populations in the southern part of the continent from more northerly peoples (Rito et al. 2013), which is followed by the split between L0d and L0k lineages (Soares et al. 2016). Although Nielsen et al. (2017) continue to suggest an East African origin for both the L0 haplotype and descendant Khoesan populations, this is based on the earlier work by Tishkoff et al. (2007, 2009) which has been superseded by the more nuanced work of Rito et al. (2013) and Chan et al. (2015). Both these latter authors strongly support a southern origin for the various subdivisions of the L0 haplotype and Rito et al. (2013) have gone so far to as to propose that when drought conditions around 115,000 years ago caused the reduction of the rainforest of central and eastern Africa, populations carrying the L0a, L0b and L0f variants were drawn north into East Africa leaving L0d and L0k populations confined to the southern coast refugia. This southern scenario was originally proposed on morphological data by Morris (2002). The more recent migration of Bantu speaking agriculturalist could also have contributed by wiping out a pre-existing diversity of L0d lineages in other regions of sub-Saharan Africa (Schlebusch et al. 2013).

The L0d haplogroup clade is largely represented in southern Africa and is assumed to have early roots going back at least 100,000 years (Schlebusch et al. 2013), perhaps around the same time as the appearance of the L1-6 clades. The subdivision of L0d1 and L0d2 as separate clades was initially thought to have occurred somewhere between 40,000 and 60,000 years ago (Schlebusch et al. 2013; Al-Hindi et al. 2022). The L0d3b clade is found amongst the Sandawe of East Africa and this has been used to argue that L0d was present in the early history of L0 in East Africa with the Sandawe representing a remnant fragment of this original group. Although it is possible to hypothesise that there was an early divergence in East Africa, Rito et al. (2013) provide a different interpretation. Since L0d3a is entirely within southern Africa, they suggest that populations carrying

L0d3a dispersed north into East Africa around 25,000 years ago and L0d3b was a divergence subsequent to that. This would suggest that click-languages were carried into East Africa relatively recently linked to a demographic post-glacial expansion from the south, and idea originally proposed by Morris (2003).

Barbieri et al. (2014a, b) have suggested that the history of Khoesan populations is more complex than previously believed and that there are both geographic and temporal patterns in the mitochondrial history in which Rito et al.'s (2013) proposal would make sense. The northwestern Khoesan populations (Kx'a language family speakers) would have been separated from the southern and south-central Kalahari groups around 35,000 years ago (Pickrell et al. 2012), and that the presence of L0k lineages is specific to northern regions (Barbieri et al. 2013). More recent exchanges between these groups would have started around 5000 years ago (Uren et al. 2016). Chan et al. (2015) have gone further in suggesting that the emergence of the major lineages L0d1a and b, L0d2a, L0d2b and L0d2d (three of which are represented in our small aDNA sample) are part of the active evolution of these haplotypes at the time of the Last Glacial Maximum between 21,000 and 17,000 years ago. Most lineages show a burst of branching events starting at 25,000 years ago, after the time of climatic deterioration and aridification of the Central Kalahari area. Climatic changes would have pushed populations in search of more suitable environments and brought further lineage differentiation (Schlebusch et al. 2013; Barbieri et al. 2013). Some sub-lineages, such as L0d2a, on the other hand, had a more recent significant expansion over the last 8,000 years. This is reinforced by Rito et al. (2013) who found that L0d underwent a demographic increase between 13,000 and 16,000 years ago. The idea of restricted population movement with subsequent genome diversification during the Last Glacial Maximum in the transKalahari region has been supported by Wang et al. (2020), Lipson et al. (2022) and Al-Hindi et al. (2022). This has echoed the explanation for distinct Khoesan morphology proposed by Morris (2002).

Very little has been written about the L0f clade except to note that it is a deep basal branch divergence that is presently centred in Uganda and Tanzania (Rito et al. 2013) and has been identified in an ancient individual at Fingura in Malawi (Lipson et al. 2022). The presence of an L0f1 individual at Faraoskop is therefore certainly of interest and at face value raises the question of how this haplotype is present amongst a group of L0d individuals in South Africa. Rito et al. (2013) have looked at the modern distribution of L0f1 and note that it is found in some southern African Bantu speakers and when found in East Africa it is at the southern edge of the range for the haplotype. They suggest that L0f1 might actually have had a southern origin but has been lost in Khoesan populations due to drift. If this is correct, then the presence of L0f1 is a remnant of the earlier distribution that was still present in the far south around 2000 years ago but has subsequently been lost.

This discussion of L0 haplotype variation in southern Africa suggests that any simple interpretation of a sequential development of haplogroups is unlikely to be correct and that the genetic history of Khoesan populations is not only deep rooted and fundamental to understanding the origin of modern humans but is also complex and needs to be better understood in the local context of southern Africa. The presence of different L0 lineages in ancient archaeological sites, both associated to foragers and pastoralists, signals the ubiquitous presence of this branch in the early history of Khoe and San groups. The Faraoskop individuals in Table 5 demonstrate at least three female lines of mitochondrial inheritance in one group. Such female lineage diversity is not surprising because female exogamy is likely to have been practiced in small foraging groups to avoid inbreeding, but Lipson et al. (2022) suggest that such gene flow might be even more complex. In that study, genome analysis performed on the FK2 and the St Helena Bay skeleton indicates that these two contemporaneous individuals were each the offspring of consanguineous unions at a first cousin level, according to homozygosity estimations. This might suggest that some groups may have been endogamous at least some of the time.

Conclusion

Substantial archaeological evidence indicates that the 12 individuals excavated from the Rock Shelter at Faraoskop were buried there in a single event some 2000 years ago. The proximity and overlap of the bodies, the close correlation of multiple radiocarbon dates, combined with reported signs of violent death (Dlamini and Morris 2024) are all supportive of this interpretation.

The three L0 mitochondrial haplotypes from the six Faraoskop individuals (L0d1, L0d2, and L0f) suggest a diversity of maternal lineages compatible with the diversity of past and present time Khoesan groups. Given the simultaneity of the burial, it is tempting to suggest an overall relatedness for the individuals examined, at least for those with similar maternal haplotypes. Whole-genome data could elucidate potential relatedness beside the maternal lineages examined.

The comparison of the mitochondrial haplotypes from Faraoskop, the two individuals in the pastoralist outgroup and other published individuals suggests that the overall mitochondrial diversity in ancient South Africa is substantial. With exception of the single L0f individual from Faraoskop, the other 12 cases of ancient mtDNA (Table 5) are all L0d variations. Similar haplotypes are present on both the

east and west coast of South Africa and within forager and pastoralist groups at the time of Faraoskop.

Future investigation of the nuclear genomes of these individuals, using optimized targeted enrichment approaches (Fu et al. 2013; Haak et al. 2015; Rohland et al. 2022), will enable deeper insights into the relationships among the studied individuals and broader connections in the genetic landscape of ancient Africa.

What is most important for understanding the genetic history of Khoesan peoples is that the analysis of so many individuals from one site provides a more focused picture than the published reports of single individuals elsewhere. If the Faraoskop sample is indeed the remains of a single mortality event, then this is a first window into the genetic dynamics of a single band of LSA foragers.

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Declarations

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References

Alder D (1988) An analysis of the human skeletal remains from Faraoskop, Western Cape, South Africa. unpublished Honours Thesis, Department of Anatomy and Cell Biology, University of Cape Town

Al-Hindi DR, Reynolds AW, Henn BM (2022) Genetic divergence within southern Africa during the Later Stone Age. In: Grine FE (ed) Hofmeyr: a Late Pleistocene human skull from South Africa. Vertebrate Paleobiology and Paleoanthropology Series. New York: Springer, New York, pp 19–28

Andrades-Valtueña A, Mittnik A, Key FM, Haak W, Allmäe R, Belinskij A, Daubaras M, Feldman M, Jankauskas R, Janković I, Massy K, Novak M, Pfrengle S, Reinhold S, Šlaus M, Spyrou MA, Szécsényi-Nagy A, Tõrv M, Hansen S, Bos KI, Stockhammer PW, Herbig A, Krause J (2017) The Stone Age Plague and Its Persistence in Eurasia. Curr Biol 27(23):3683-3691.e8. https://doi.org/10.1016/j.cub.2017.10.025

Andrews RM et al (1999) Reanalysis and revision of the Cambridge referencesequence for human mitochondrial DNA. Nat Genet 23:147

Barbieri C, Vicente M, Rocha J, Mpoloka SW, Stoneking M, Pakendorf B (2013) Ancient Substructure in Early mtDNA Lineages of Southern Africa. Amer J Hum Gen 92:285–292

Barbieri C, Güldemann T, Naumann C, Gerlach L, Berthold F, Nakagawa H, Mpoloka SW, Stoneking M, Pakendorf B (2014a) Unraveling the complex maternal history of Southern African Khoisan populations. Amer J Phys Anthropol 153(3):435–448. https://doi.org/10.1002/ajpa.22441

Barbieri C, Vicente M, Oliveira S, Bostoen K, Rocha J, Stoneking M, Pakendorf B (2014b) Migration and interaction in a contact zone: mtDNA variation among Bantu-speakers in southern Africa. PLoS ONE 9(6):e99117. https://doi.org/10.1371/journal.pone.0099117

Behar DM, Villems R, Soodyall H, Blue-Smith J, Pereira L, Metspalu E, Scozzari R, Makkan H, Tzur S, Comas D, Bertranpetit J, Quintana-Murci L, Tyler-Smith C, Wells RS, Rosset S (2008) The dawn of human matrilineal diversity. Amer J Hum Gen 82:1130–1140. http://www.cell.com/AJHG/abstract/S0002-9297(08)00255-3

Brucato N, Fernandes V, Mazières S, Kusuma P, Cox MP, Ng'ang'a JW, Omar M, Simeone-Senelle M-C, Frassati C, Alshamali F, Fin B, Boland A, Deleuze J-F, Stoneking M, Adelaar A, Crowther A, Boivin N, Pereira L, Bailly P, … Ricaut F-X (2018) The comoros show the earliest austronesian gene flow into the swahili corridor. Amer J Hum Gen 102(1):58–68. https://doi.org/10.1016/J.AJHG.2017.11.011

Cameron ME, Pfeiffer S, Stock J (2021) Small body size phenotypes among middle and later stone age southern Africans. J Hum Evol 152:102943

Chan EKF, Hardie R-A, Petersen DC, Beeson K, Bornman RMS, Smith AB et al (2015) Revised Timeline and Distribution of the Earliest Diverged Human Maternal Lineages in Southern Africa. PLoS ONE 10(3):e0121223. https://doi.org/10.1371/journal.pone.0121223

Chan EKF, Timmermann A, Baldi BF, Moore AE, Lyons RJ, Lee S-S, Kalsbeek AMF, Petersen DC, Rautenbach H, Förtsch HEA, Bornman MSR, Hayes VM (2019) Human origins in a southern African palaeo-wetland and first migrations. Nature 575:185–189. https://doi.org/10.1038/s41586-019-1714-1

Dabeny J, Knapp M, Glocke I, Gansauge M-T, Weihmann A, Nickel B, Valdiosera C, Garcia N, Pääbo S, Arsuaga J-L, Meyer M (2013) Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments.

PNAS 110(39):15758–15763. https://doi.org/10.1073/pnas.1314445110

Diallo MY, Čížková M, Kulichová I, Podgorná E, Priehodová E, Nováčková J, Fernandes V, Pereira L, Černý V (2022) Circum-Saharan Prehistory through the Lens of mtDNA Diversity. Genes 13(3):533. https://doi.org/10.3390/genes13030533

Dlamini N, Morris AG (2024) Peri-mortem cranial trauma: implications for violent deaths at the Faraoskop rock shelter. Archaeol Anthropol Sci 17(6). https://doi.org/10.1007/s12520-024-02118-x

Fu Q, Meyer M, Gao X, Pääbo S (2013) DNA analysis of an early modern human from Tianyuan Cave, China. Proc Natl Acad Sci U S A 110:2223–2227

Gibbon V, Paximandis M, Strakalj G, Ruff P, Penny C (2009) Novel methods of molecular sex identification from skeletal tissue using the amelogenin gene. Forensic Sci Int-Gen 3(2):74–79. https://doi.org/10.1016/j.fsigen.2008.10.007

Gonder MK, Mortensen HM, Reed FA, de Sousa A, Tishkoff SA (2007) Whole-mtDNA genome sequence analysis of ancient African lineages. Mol Biol Evol 24:757–768. https://doi.org/10.1093/molbev/msl209

Gray K, le Roux P, Sealy J (n.d.) (this volume) Diet and landscape use at Faraoskop from C, N and Sr isotopes in multiple skeletal tissues. Archaeol Anthropol Sci. https://doi.org/10.1007/s12520-024-02085-3

Haak W, Lazaridis I, Patterson N, …Reich D. (2015) Massive migration from the steppe was a source for Indo-European languages in Europe. Nature 522:207–211

Henn BM, Gignous CR, Jobin M, Granka JM, Macpherson JM, Kidd JM, Rodriguez-Botigué L, Ramachandran S, Hon L, Brisbin A, Lin AA, Underhill PA, Comas D, Kidd KK, Norman PJ, Parham P, Bustamante CD, Mountain JL, Feldman MW (2011) Hunter-gatherer genomic diversity suggests a southern African origin for modern humans. PNAS 108(13):5154–5162

Irish JD, Black W, Sealy J, Ackermann RR (2014) Questions of Khoesan continuity: dental affinities among the indigenous Holocene peoples of South Africa. Am J Phys Anthropol 155:33–44

Jónsson H, Ginolhac A, Schubert M, Johnson PLF, Orlando L (2013) MapDamage20: fast approximate Bayesian estimates of ancient DNA damage parameters. Bioinformatics 29(13):1682–1684

Kim KY, Kwon Y, Bazarragchaa M, Kim K (2013) A real-time PCR-based amelogenin Y allele dropout assessment model in gender typing of degraded DNA samples. Int J Legal Med 127:55–61. https://doi.org/10.1007/s00414-011-0663-5

Kircher M, Sawyer S, Meyer M (2012) Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. Nucleic Acids Res 40:e3

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760

Lipson M, Sawchuk EA, Thompson JC, … Prendergast ME (2022) Ancient DNA and deep population structure in sub-Saharan African foragers. Nature 603:290–296. https://doi.org/10.1038/s41586-022-04430-9

Manhire T (1993) A report on the excavation at Faraoskop Rock Shelter in the Graafwater district of the South-Western Cape. S Afr Field Archaeol 2(1):2–23

Maricic T, Whitten M, Pääbo S (2010) Multiplexed DNA sequence capture of mitochondrial genomes using PCR products. PLoS ONE 5:e14004

Merheb M, Matar R, Hodeify R, Siddiqui SS, Vazhappilly CG, Marton J, Azharuddin S, Zouabi HAL (2019) Mitochondrial DNA, a powerful tool to decipher ancient human civilization from domestication to music, and to uncover historical murder cases. Cells 8(5):433. https://doi.org/10.3390/CELLS8050433

Meyer M, Kircher M (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harbor Protocols. Jun;2010(6):pdb.prot5448.

Mittnik A, Wang CC, Svoboda J, Krause J (2016) A Molecular Approach to the Sexing of the Triple Burial at the Upper Paleolithic Site of Dolní Věstonice. PLoS ONE 11(10):e0163019. https://doi.org/10.1371/journal.pone.0163019

Morris AG (2002) Isolation and the origin of the Khoisan: Late pleistocene and early holocene human evolution at the Southern end of Africa. Hum Evol 17(3–4):231–240

Morris AG (2003) The myth of the East African 'Bushmen.' S Afr Archaeol Bull 58(178):85–90

Morris AG, Heinze A, Chan EKF, Smith AB, Hayes VM (2014) First ancient mitochondrial human genome from a prepastoralist southern African. Genome Biol Evol 6:2647–2653. https://doi.org/10.1093/gbe/evu202

Morris AG, Dlamini N, Joseph J, Parker A, Powrie C, Ribot I, Stynder D (2005) Later stone age burials from the western cape province, South Africa, Part 1: Voëlvlei. S Afr Field Archaeol 13(14):19–26

Nielsen R, Akey JM, Jakobsson M, Pritchard JK, Tishkoff S, Willersev E (2017) Tracing the peopling of the world through genomics. Nature 541:302–310. https://doi.org/10.1038/nature21347

Oliveira S, Fehn A-M, Aço T, Lages F, Gayà-Vidal M, Pakendorf B, Stoneking M, Rocha J (2018) Matriclans shape populations: Insights from the Angolan Namib Desert into the maternal genetic history of southern Africa. Am J Phys Anthropol 165(3):518–535. https://doi.org/10.1002/ajpa.23378

Pakendorf B, Stoneking M (2005) Mitochondrial DNA and human evolution. Annu Rev Genomics Hum Genet 6(1):165–183. https://doi.org/10.1146/annurev.genom.6.080604.162249

Paradis E (2010) pegas: an R package for population genetics with an integrated–modular approach. Bioinformatics 26:419

Paradis E, Claude J, Strimmer K (2004) APE: Analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290. https://doi.org/10.1093/bioinformatics/btg412

Parkington J, Dlamini N (2015) First People: ancestors of the San. Cape Town: Creda Communications

Peltzer A et al (2016) EAGER: efficient ancient genome reconstruction. Genome Biol 17:60

Pfeiffer S, Harrington L, Lombard M (2019) The people behind the samples: Biographical features of past hunter-gatherers from KwaZulu-Natal who yielded aDNA. Int J Paleopathol 24:158–164. https://doi.org/10.1016/j.ijpp.2018.10.008

Pfeiffer S, Sealy J, Harrington L, Loftus E, Maggs T (2020) A late Holocene community burial area: Evidence of diverse mortuary practices in the Western Cape. South Africa Plos One 15(4):e0230391

Pickrell JK, Patterson N, Barbieri C, Berthold F, Gerlach L, Lipson M, Po-Ru L, Lachance J, Güldemann T, Kure B, Wata SM, Nakagawa H, Naumann C, Mountain JL, Bustamante CD, Berger B, Stoneking M, Reich D, Pakendorf B (2012) The genetic prehistory of southern Africa. Nat Commun 3. https://doi.org/10.1038/ncomms2140

Pinhasi P, Fernandes D, Sirack K, … Hofreiter, M (2015) Optimal ancient DNA yields from the inner ear part of the human petrous bone. PloS One 10:e0129102

Reich D (2019) Who we are and how we got here: ancient DNA and the new science of the human past. Oxford: Oxford University Press

Renaud G, Slon V, Duggan AT, Kelso J (2015) Schmutzi: an estimation of contamination and endogenous mitochondrial consensus calling for ancient DNA. Genome Biol 16:244. https://doi.org/10.1186/s13059-015-0776-0

Rito T, Richards MB, Fernandes V, Alshamali F, Cerny V et al (2013) The first modern human dispersals across Africa. PLoS ONE 8(11):e80031. https://doi.org/10.1371/journal.pone.0080031

Rohland N, Mallik S, Mah M, Maier R, Patterson N, Reich D (2022) Three assays for in-solution enrichment of ancient human DNA at more than a million SNPs. Genome Res 32:2068–2078

Sawyer S, Krause J, Guschanski K, Savolainen V, Paabo S (2012) Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA. PLoS ONE 7:e34131

Schlebusch CM, Lombard M, Soodyall H (2013) MtDNA control region variation affirms diversity and deep substructure in populations from southern Africa. BMC Evol Biol 13:56

Schlebusch CM, Malmström H, Günther T, Sjödin P, Coutinho A, Edlund H, Munters A, Vicente V, Steyn M, Soodyall H, Lombard M, Jakobsson M (2017) Southern African ancient genomes estimate modern human divergence to 350,000 to 260,000 years ago. Science 358:652–655. https://doi.org/10.1126/science.aao6266

Schlebusch CM, Sjödin P, Breton G, Günther T, Naidoo T, Hollfelder N, Sjöstrand AE, Xu J, Gattepaille LM, Vicente M, Scofield DG (2020) Khoe-San genomes reveal unique variation and confirm the deepest population divergence in Homo sapiens. Mol Biol Evol 37:2944–2954

Schönherr S, Weissensteiner H, Kronenberg F, Forer L (2023) (2023) Haplogrep 3 - an interactive haplogroup classification and analysis platform. Nucleic Acids Res 51(W1):W263–W268. https://doi.org/10.1093/nar/gkad284

Sealy JC, Patrick MK, Morris AG, Alder D (1992) Diet and dental caries among later stone age inhabitants of the Cape Province, South Africa. Am J Phys Anthropol 88:123–143

Sirak K, Fernandes D, Cheronet O, … Pinhasi R (2020) Human auditory ossicles as an alternative optimal source of ancient DNA. Genome Res 30:427–436

Skoglund P, Stora J, Götherström A, Jakobsson M (2013) Accurate sex identification of ancient human remains using DNA shotgun sequencing. J Archaeol Sci 40:4477–4482

Skoglund P, Thompson JC, Prendergast ME, Reich D (2017) Reconstructing Prehistoric African Population Structure. Cell 171. https://doi.org/10.1016/j.cell.2017.08.049

Smith AB (2006) Excavations at Kasteelberg and the origins of the Khoekhoen in the Western Cape, South Africa. BAR International Series, No 1537. Cambridge Monographs in African Archaeology

Soares P, Alshamali F, Pereira JB, Fernandes V, Silva NM, Afonso C, Costa MD, Musilova E, Macaulay V, Richards MB, Cerny V, Pereira L (2012) The Expansion of mtDNA Haplogroup L3 within and out of Africa. Mol Biol Evol 29(3):915–927. https://doi.org/10.1093/molbev/msr245

Soares P, Rito T, Pereira L, Richards MB (2016) A genetic perspective on African prehistory. In: Jones SC, Stewart BS (eds), Africa from MIW 6–2: Population Dynamics and Paleoenvironments, Chapter 18:383–405. Vertebrate Paleobiology and Paleoanthropology Series, Netherlands: Springer

Stynder DD, Ackermann RR, Sealy JC (2007) Early to mid-holocene South African later stone age human crania exhibit a distinctly Khoesan morphological pattern. S Afr J Sci 103:349–352

Tishkoff SA, Gonder MK, Henn BM, Mortensen H, Knight A, Gignoux C, Fernandopulle N, Lema G, Nyambo TB, Ramakrishnan U, Reed FA, Mountain JL (2007) History of Click-Speaking Populations of Africa Inferred from mtDNA and Y Chromosome Genetic Variation. Molec Biol Evol 24(10):2180–2195. https://doi.org/10.1093/molbev/msm155

Ubelaker DH (1978) Estimating sex, stature and age. In: Ubelaker DH (ed) Skeletal remains: Excavation, analysis, interpretation. Aldine Press, Chicago, pp 41–67

Uren C, Kim M, Martin AR, Bobo D, Gignoux CR, van Helden PD, Möller M, Hoal EG, Henn BM (2016) Fine-scale human population structure in Southern Africa reflects ecogeographic boundaries. Genetics 204:303–314. https://doi.org/10.1534/genetics.116.187369

Vicente M, Schlebusch CM (2020) African population history: an ancient DNA perspective. Curr Opin Genet Dev 62:8–15. https://doi.org/10.1016/J.GDE.2020.05.008

Wang K, Goldstein S, Bleasdale M et al. (2020) Ancient genomes reveal complex patterns of population movement, interaction, and replacement in sub-Saharan Africa. Sci Adv 6:eaaz0183. https://doi.org/10.1126/sciadv.aaz0183

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York. https://ggplot2.tidyverse.org