

HUMAN EVOLUTION

Southern African ancient genomes estimate modern human divergence to 350,000 to 260,000 years ago

Carina M. Schlebusch,^{1,4*} Helena Malmström,^{1,4*} Torsten Günther,¹ Per Sjödin,¹ Alexandra Coutinho,¹ Hanna Edlund,¹ Arielle R. Munters,¹ Mário Vicente,¹ Maryna Steyn,² Himla Soodyall,³ Marlize Lombard,^{4,5†} Mattias Jakobsson^{1,4,6†}

Southern Africa is consistently placed as a potential region for the evolution of *Homo sapiens*. We present genome sequences, up to 13x coverage, from seven ancient individuals from KwaZulu-Natal, South Africa. The remains of three Stone Age hunter-gatherers (about 2000 years old) were genetically similar to current-day southern San groups, and those of four Iron Age farmers (300 to 500 years old) were genetically similar to present-day Bantu-language speakers. We estimate that all modern-day Khoe-San groups have been influenced by 9 to 30% genetic admixture from East Africans/Euradians. Using traditional and new approaches, we estimate the first modern human population divergence time to between 350,000 and 260,000 years ago. This estimate increases the deepest divergence among modern humans, coinciding with anatomical developments of archaic humans into modern humans, as represented in the local fossil record.

Archaeological, fossil, and genetic data place early traces of anatomically modern humans in sub-Saharan Africa (1, 2). The earliest fully modern human remains, dating to ~190 thousand years ago (ka), originate from Ethiopia (1), and fossils displaying some features of early anatomical modernity from Morocco are dated to ~315 ka (3).

Southern Africa has been occupied by the genus *Homo* from about 2 million years ago (Ma), with a major transitional phase from the Earlier to the Middle Stone Age, between 600 ka and 200 ka (4). The fossil record indicates the presence of archaic *H. sapiens* at >200 ka and anatomically modern humans from ~120 ka (5). Genetic studies identified southern African Khoe-San populations as carrying more unique variants and more divergent lineages than other living groups (6–10). The deepest population split among modern humans—between Khoe-San and other groups—was estimated to ~160 to 100 ka on the basis of short-sequence fragments (7) and genome-wide single-nucleotide polymorphism data (6). Rescaled estimates range between 250 and 300 ka after re-

visions of the human mutation rate from pedigrees (11). Genetic variation in the Khoe-San was used previously to argue for a southern African origin of modern humans (9), although multiple regions in Africa have been proposed also (6, 12).

Middle Stone Age sites in KwaZulu-Natal, South Africa, demonstrate human occupation since >100 ka (4). We report on the genomes of seven ancient individuals from KwaZulu-Natal (Table 1 and Fig. 1). We sequenced three Stone Age hunter-gatherers and four Iron Age farmers, dated to ~2 ka and 0.5 to 0.3 ka, respectively, to between 0.01x and 13.2x genome coverage (Fig. 1 and Table 1) (13). The data display characteristics of ancient DNA (13).

The three Stone Age individuals—Ballito Bay A, Ballito Bay B, and Doonside—and an Iron Age individual from Champagne Castle carry mitochondrial subhaplogroups belonging to L0d (13), common in current-day Khoe-San (10). The remaining three Iron Age individuals—from Newcastle, Eland Cave, and Mfongosi—have mitochondrial DNA haplogroups that fall within L3e, common in current-day Bantu-language-speaking groups (10). Both males from Ballito Bay carry the Y chromosome Al1b2 haplotype (13), common among modern-day Khoe-San (10). All seven individuals exhibited non-lactase persistence variants (table S25). Of the Iron Age individuals, three carry at least one Duffy null allele, protecting against malaria, and two have at least one sleeping-sickness-resistance variant in the *APOLI* gene (13). The Stone Age individuals do not carry these protective alleles (13) (table S25).

To assess population affinities among the ancient individuals and their relations to modern-day groups, we merged the ancient genome data with genotype data sets from southern Africa (6, 8), Africa, and across the globe (13) (table S8). We further merged our data with a set of complete genomes of 11 individuals from across the

world, including southern, eastern, and western Africa, as well as with that of Neandertals and Denisovans (13, 14). Principal components analysis (PCA) and admixture analyses show that the Stone Age individuals are related to present-day southern Khoe-San (Fig. 1, B and C, figs. S4 to S8, and figs. S29 to S32). The Iron Age individuals group with populations of West African descent and are closest related to current southeast Bantu-language speakers from South Africa. They display similar levels of Khoe-San admixture (16 versus 19%) (Fig. 1, B and C, and figs. S4 to S9), consistent with archaeological evidence for Iron Age farmers arriving in eastern southern Africa by ~1.7 ka (15). Among western Bantu-language speakers, they cluster in particular with groups from Angola, supporting the late-split linguistic hypothesis (13, 16).

The Stone Age individuals form one extreme in the PCA, separating Khoe-San from other Africans and non-Africans (Fig. 1B) (13). Modern-day Khoe-San, including Ju|'hoansi, are drawn toward other Africans and non-Africans compared with the Stone Age individuals from Ballito Bay (6, 8). Although low levels of admixture from other African groups into Khoe-San groups were suggested (6, 8), it has been difficult to estimate its magnitude and impact, due to lack of a less- or nonadmixed San reference.

We tested various admixture scenarios into Khoe-San groups using the 13x coverage, high-quality genome [Uracil-DNA-glycosylase treated (13)] of Ballito Bay A. Our results show that the post-2 ka admixture source in modern-day Khoe-San was an already-admixed Eurasian/East African group (31/69%) comparable to the Amhara in eastern Africa (fig. S12 and tables S9 and S15). We estimate that the Ju|'hoansi (historical foragers) received 9 to 14%, the Nama (historical herders) received 23 to 30%, and all modern-day Khoe-San contain 9 to 30% admixture from this Eurasian/East African group (Fig. 2 and table S15) (13). We dated the admixture event to 50 ± 3 and 44 ± 4 generations ago for the Ju|'hoansi and Nama, respectively, corresponding to 1.5 and 1.3 ka [assuming 30 years per generation (13)], consistent with a migration of East African pastoralists admixing with local Stone Age hunter-gatherers ≥1.5 ka (6, 17, 18). This admixture resulted in elevated diversity in present-day Khoe-San groups (Fig. 3 and fig. S16) (13), generally lower levels of runs of homozygosity in Khoe-San compared with Ballito Bay A (Fig. 2B), and inflated estimates of past effective population sizes (19, 20) (fig. S18). It is likely that Khoe-San groups harbor the greatest level of diversity due to capturing the deepest split among humans combined with recent admixture and that their (census) population size in prehistory has not been much greater compared with other African groups [in contrast to (21)].

To decipher early human history, we used several complementary approaches (7, 19). We focus on the 13x coverage genome of Ballito Bay A, a hunter-gatherer boy who was unaffected by admixture with herders from East Africa, Bantu-speaking farmers from West Africa, or Eurasian immigrants.

¹Department of Organismal Biology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18C, SE-752 36 Uppsala, Sweden. ²Human Variation and Identification Research Unit, School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, South Africa. ³Division of Human Genetics, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand and National Health Laboratory Service, Johannesburg, South Africa. ⁴Centre for Anthropological Research and Department of Anthropology and Development Studies, University of Johannesburg, Post Office Box 524, Auckland Park, 2006, South Africa. ⁵Stellenbosch Institute for Advanced Study (STIAS), Wallenberg Research Centre at Stellenbosch University, Marais Street, Stellenbosch, 7600, South Africa. ⁶Science for Life Laboratory (SciLife Lab), Uppsala, Sweden.

*These authors contributed equally to this work. †Corresponding author. Email: mattias.jakobsson@ebc.uu.se (M.J.); mlombard@uj.ac.za (M.L.)

We estimated divergences between various sets of individuals on the basis of diploid called sites of Ballito Bay A (13) and 12 high-coverage modern and archaic human genomes (14), using a coalescent-based approach [G-PhoCS (7, 13)], assuming 1.25×10^{-8} mutations per generation (11) and 30 years per generation. We estimate the split times between Ballito Bay A and modern-day individuals (excluding Ju|'hoansi) to between 285 and 356 ka, with the deepest split time to 356 ± 7 ka for comparison with the Mandenka of West Africa (Fig. 3 and figs. S19 and S20) (13). Population split times

with the admixed Ju|'hoansi instead of Ballito Bay A are on average 55,000 years younger (fig. S20 and table S22). Although intrinsic properties of ancient DNA may contribute to this difference, the greater effect likely comes from the fact that the Ju|'hoansi display 9 to 14% admixture with East Africans/Euradians (13).

We developed a method [two plus two (TT) (13)] that alleviates assumptions about past population sizes and is robust to low levels of admixture, using the concordance approach (6). Assuming a general split model without migration, and pick-

ing two chromosomes (from two individuals or from one individual with diploid data) from each of two subpopulations, it is possible to derive the parameters from the frequencies of the eight possible polymorphic sample configurations (assuming sequence data, an infinite-sites model, and a known ancestral variant state). We estimate the population split time separately for each branch in a two-population model, resulting in two estimates of the same split-time parameter. It provides the possibility to estimate, independently, the split between Ballito Bay A and other groups, with genetic data from modern-day individuals avoiding bias caused by properties of ancient DNA, and the need for phased data. We evaluate this approach, demonstrating that split-time estimates are accurate, little affected by low levels of admixture/migration (13), and improve with genome coverage (13).

For the Ballito Bay A versus Dinka split, the two branches are estimated to 301 ± 5 ka and 265 ± 5 ka, respectively (Fig. 3B and fig. S24) (13), demonstrating the deepest split at >260 ka (even based on the Dinka's genetic variation to avoid possible impact of ancient DNA properties (13)). The Ju|'hoansi versus Dinka split has similar split times for the two estimated branches (258 ± 5 ka for Ju|'hoansi and 255 ± 5 ka for Dinka) (Fig. 3B and fig. S24) (13), thus 43 to 10 ka less than the Ballito Bay A versus Dinka split, a difference likely due to the admixture from East Africans/Euradians into present-day Khoes-Sans (13). Thus, our results show that the deepest split among modern humans (the estimated latest time for the emergence of *H. sapiens*) occurred at between 350 and 260 ka (Fig. 3). Additional gene flow between southern African hunter-gatherers and other groups >2 ka would only lead to our dates being underestimates for the true population split time. This deep divergence is halfway to the human versus Neandertal/Denisovan split (Fig. 3 and table S24) (13, 14) and as deep as that estimated between the Denisovans and Neandertals (2, 14).

We acknowledge that mutation-rate estimates are debated, based on methodology. A consensus rate of 1.25×10^{-8} per base pair per generation has emerged (11), but this might be revised, affecting the chronological dating of events inferred

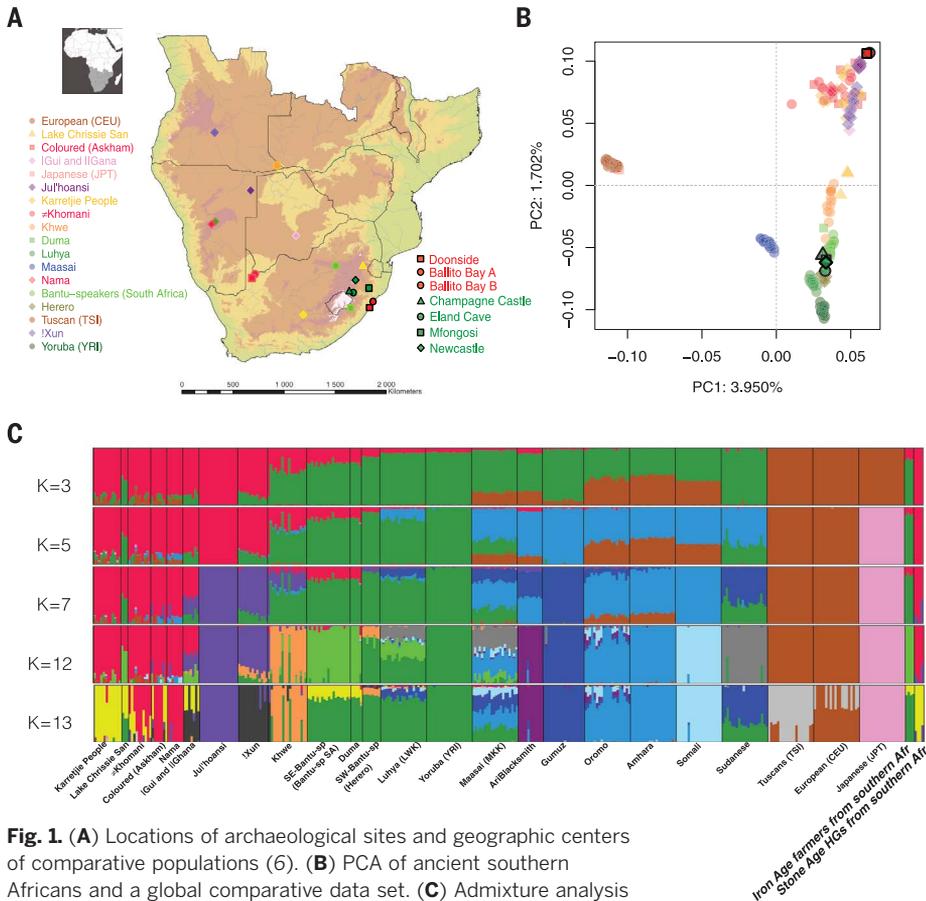


Fig. 1. (A) Locations of archaeological sites and geographic centers of comparative populations (6). (B) PCA of ancient southern Africans and a global comparative data set. (C) Admixture analysis for selected clusters (K); see fig. S8 for all K values.

Table 1. Sample information with calibrated dates, genomic and mitochondrial sequence coverage, sex, and mitochondrial and Y-chromosomal haplogroups [see (13)]. B.P., before the present.

Sample	Calibrated date B.P. (2 SD)	Genomic DNA coverage	Mitochondrial DNA coverage	Biological sex determination	Mitochondrial haplogroup	Y-chromosome haplogroup	Morphological sex determination
Ballito Bay A	1986–1831*	12.94	1035	XY	L0d2c1	A1b1b2	Juvenile
Ballito Bay B	2149–1932	1.25	84	XY	L0d2a1	A1b1b2	Male
Doonside	2296–1910*	0.01	2.6	–	L0d2	–	–
Champagne Castle	448–282	0.36	186	XX	L0d2a1a	–	Female
Eland Cave	533–453	13.23	7597	XX	L3e3b1	–	Female
Mfongosi	448–308	6.94	562	XX	L3e1b2	–	Female
Newcastle	508–327	10.65	616	XX	L3e2b1a2	–	Female

*Dated by Ribot et al. (15).

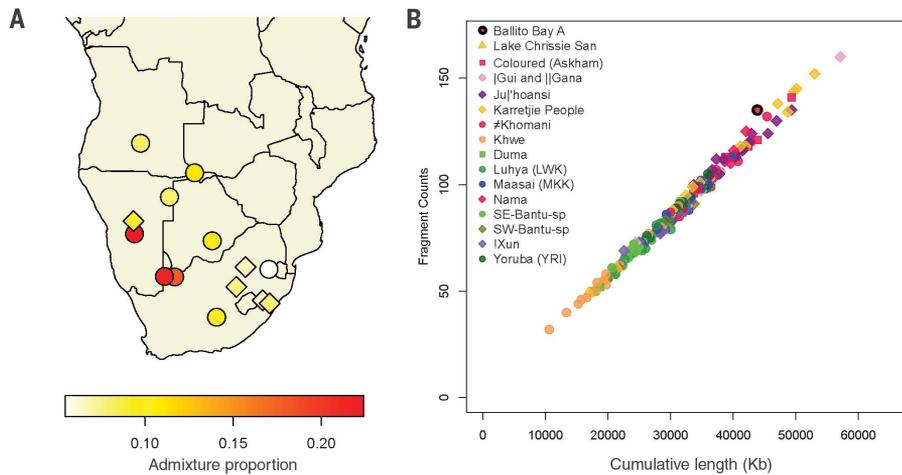


Fig. 2. (A) East African/Eurasian admixture proportions (f_4 ratio test using Amhara as the reference population) for current-day southern African populations (6). Circles depict San and Khoekhoe populations, and diamonds depict Bantu-language speakers. **(B)** Runs of homozygosity of the 200 to 500 Kb bin among individuals from various African populations.

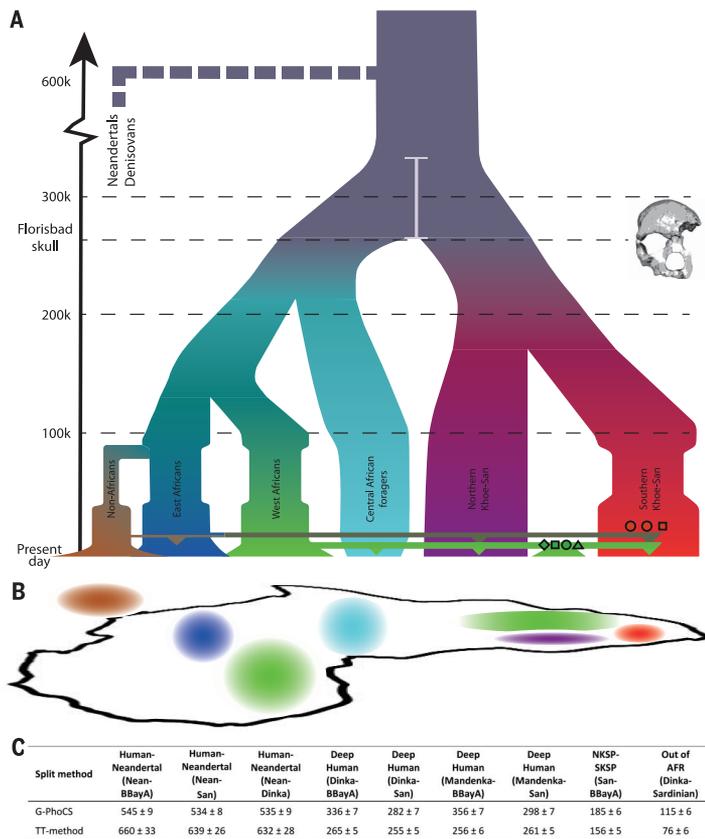


Fig. 3. Demographic model of African history and estimated divergences. (A) Population split times, hierarchy, and population sizes (width along a horizontal axis for populations) (summary of results in figs. S6, S12, S18 to S20, and S24). Horizontal colored lines represent migration, with down-pointing triangles representing admixture into another group. The estimated age of the Florisbad skull (24) is indicated by a dashed line. Stone Age hunter-gatherers are shown by red symbols, and Iron Age farmers as green symbols. **(B)** Non-Africans (brown), East Africans (blue), West Africans (green), central African hunter-gatherers (light blue), northern Khoe-San (NKSP) (purple), and southern Khoe-San (SKSP) (red) are mapped according to their broad historical distribution. **(C)** Estimated population split times with the Gronau *et al.* approach (7) and the TT method described in (13). Divergence-time estimates for human versus Neandertal and non-Africans versus Africans (out-of-Africa event) are given for reference and are consistent with recent estimates (2). NKSP-SKSP split is the estimated split time between Ballito Bay A and Ju|'hoansi.

by genomic data. Thus, although our assumptions on mutation rates and generation times influence the exact chronological estimates, our results notably increase the time depth for the deepest split for modern humans on a relative scale (illustrated by the deeper split time observed for Ballito Bay A compared with Ju|'hoansi with two independent methods).

Several studies point to the possibility of deep population structure, or archaic admixture, among sub-Saharan groups from central and West Africa (2, 22, 23). It is possible that some fraction of the deep split times between Ballito Bay A (and modern Ju|'hoansi) and modern-day sub-Saharan individuals/groups can be explained by low levels of deep structure/admixture. Unless it is common to all non-San groups, it is unlikely to have a substantial effect on the split-time estimates in this study. Within West African groups, we note a history of deep population structure (fig. S12), consistent with the larger estimates of their effective population size (fig. S21).

The San, often represented by Ju|'hoansi, has consistently been included in influential investigations on human evolutionary history as a nonadmixed population (2, 6, 9, 14), to date the deepest splits among modern humans, to infer admixture patterns of archaic humans outside of Africa, and to study the population history of sub-Saharan Africa. Many of these inferences may be biased by the substantial admixture into all Khoe-San groups that we have demonstrated here.

The deep split-time estimation of 350 to 260 ka is consistent with the archaeological estimate for the onset of the Middle Stone Age across sub-Saharan Africa (4) and coincide with archaic *H. sapiens* in southern Africa represented by, for example, the Florisbad skull dating to 259 ± 35 ka (24) and *H. naledi* dating to ~236 to 335 ka (25). Although we do not rule out that the ancestors of KwaZulu-Natal Stone Age hunter-gatherers might have originated elsewhere in sub-Saharan Africa, or might have mixed with other groups >2 ka, we suggest that archaeological, fossil, and genetic records increasingly point toward a modern human development that includes southern Africa.

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paper, with input from all authors. M.L. and M.J. were co-principal investigators on the project. Data are available from the European Nucleotide Archive (EBI-ENA) under accession no. PRJEB22660.

SUPPLEMENTARY MATERIALS

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 Materials and Methods
 Supplementary Text
 Figs. S1 to S34
 Tables S1 to S25
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Ancient DNA pushes human emergence back

Anatomically modern humans evolved in Africa, but pinpointing when has been difficult. Schlebusch *et al.* sequenced three ancient African genomes from the Stone Age, about 2000 years old, and four from the Iron Age, 300 to 500 years old. One of the oldest samples, sequenced to 13× coverage, appears most closely to resemble individuals from the present-day San population. However, this individual seems to have lacked genetic contributions from other modern African populations, including pastoralists and farmers, which were observed in modern San individuals. Thus, the earliest divergence between human populations may have occurred 350,000 to 260,000 years ago.

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