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

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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. Three Stone Age hunter-gatherers (about 2000 years old) were genetically similar to current-day southern San groups, while four Iron Age farmers (300 to 500 years old) were genetically similar to present-day Bantu-speakers. We estimate that all modern-day Khoe-San groups have been influenced by 9 to 30% genetic admixture from East Africans/Eurians. 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 kya (kya = thousand years ago/old), originates from Ethiopia (1), and fossils displaying some features of early anatomical modernity from Morocco are dated to ~315 kya (3).

Southern Africa has been occupied by the genus *Homo* from about 2 Mya, with a major transitional phase, from the Earlier to the Middle Stone Age, between 600 kya and 200 kya (4). The fossil record indicates the presence of archaic *H. sapiens* at >200 kya and anatomically modern humans from ~120 kya (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 ~160–100 kya, on the basis of short-sequence fragments (7) and genome-wide SNP data (6). Rescaled estimates range between 250 and 300 kya after revisions 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 kya (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 kya and 0.5–0.3 kya 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 sub-haplogroups belonging to L0d (13), common in current-day Khoe-San (10). The remaining three Iron Age individuals, from Newcastle, Eland Cave, and Mfongosi, have mtDNA haplogroups that fall within L3e, common in current-day Bantu-speaking groups (10). Both males from Ballito Bay carry the Y chromosome A1b1b2 haplotype (13), common among modern-day Khoe-San (10). All our 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 *APOL1* 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 archaic humans (13, 14). Principal Component 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, are closest related to current southeast Bantu speakers from South Africa and 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 kya (15). Among western Bantu-speakers, they group in particular with Bantu-speakers 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 are drawn toward other Africans and non-Africans compared to the Stone Age individuals from Ballito Bay, including Ju|'hoansi (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/non-admixed 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 kya admixture source in modern-day Khoe-San was an already-admixed Eurasian/East African group (31/69%) comparable to the Amhara (fig. S12 and tables S9 and S15). We estimate that the Ju|'hoansi (historical foragers) received 9-14%, the Nama (historical herders) received 23-30%, and all modern-day Khoe-San contain 9-30% admixture from this 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 1.5-1.3 kya [assuming 30 years/generation (13)], consistent with a migration of East African pastoralists admixing with local Stone Age hunter-gatherers ≥ 1.5 kya (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 to 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 to other African groups [contra (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. 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/generation (11) and 30 years/generation. We estimate the split-times between Ballito Bay A and modern-day individuals (excluding Ju|'hoansi) to 285-356 kya, and the deepest split-time to 356 ± 7 kya 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 9-14% admixture with East Africans/Eurians (13).

We developed a method [Two-by-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 picking two chromosomes (from two individuals or from one individual with diploid data) from each of two sub-populations, 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 kya and 265 ± 5 kya respectively (Fig. 3B and fig. S24) (13), demonstrating a deepest split at >260 kya (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 kya, Ju|'hoansi and 255 ± 5 kya, Dinka, Fig. 3B and fig. S24 (13)), thus 43-10 kya less than the Ballito Bay A versus Dinka split, a difference likely due to the admixture from East Africans/Eurians into present-day Khoe-San (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 kya and 260 kya (Fig. 3). Additional gene-flow between southern African hunter-gatherers and other groups >2 kya 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 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 to 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 'non-admixed' 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 San groups that we have demonstrated here.

The deep split-time estimation of 350-260 kya is consistent with the archaeological estimate for the onset of the Middle Stone Age across sub-Saharan Africa (4), the oldest known archaic *H. sapiens* represented by the Florisbad skull dating to 259 ± 35 kya (24), and *H. naledi* of ~236-335 kya (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 kya, we suggest that archaeological, fossil, and genetic records increasingly point toward a modern human development that includes southern Africa.

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Supplementary Text

Figs. S1 to S34

Tables S1 to S25

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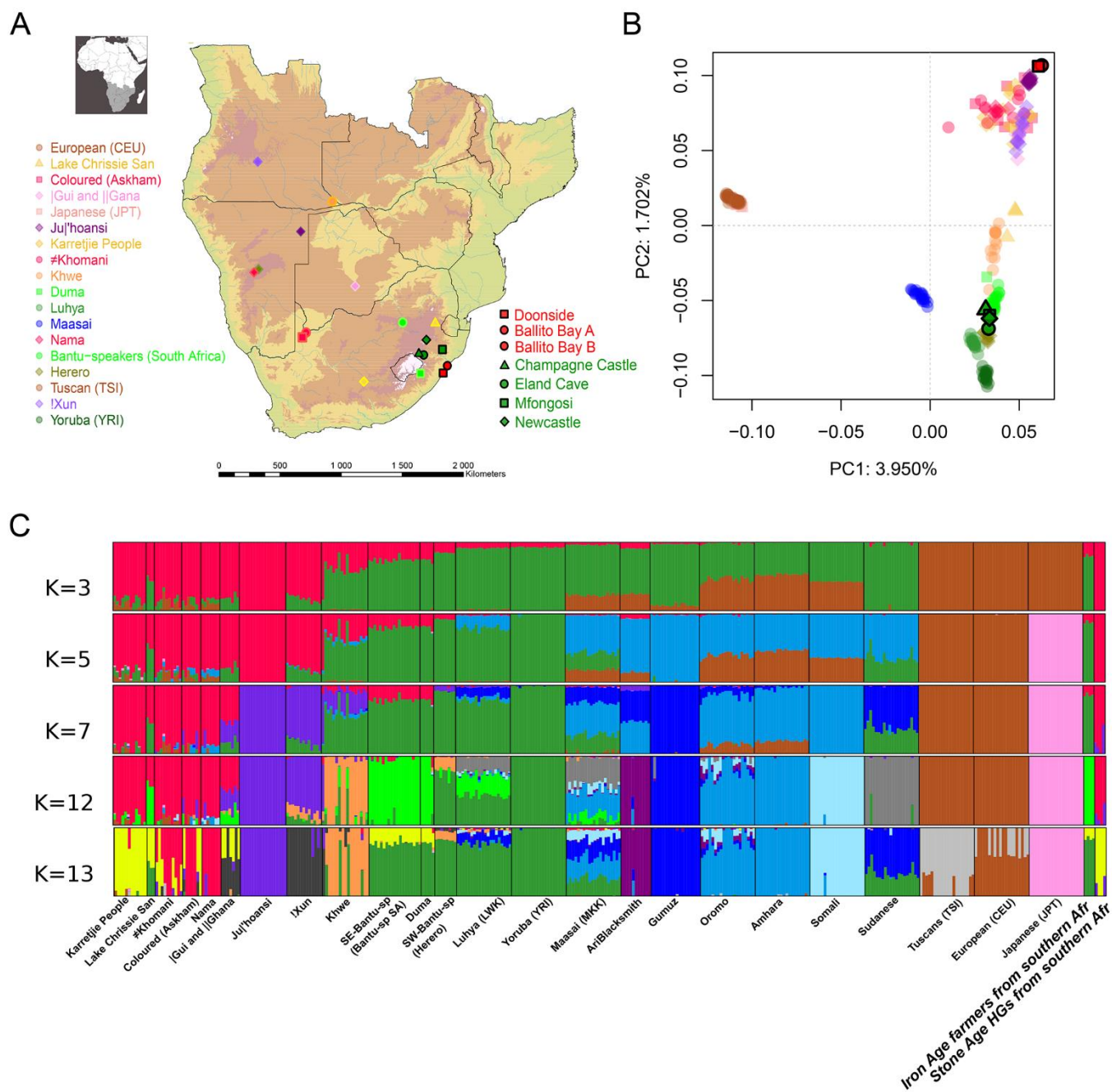


Fig. 1. (A) Locations of archaeological sites and geographic centers of comparative populations (6). (B) PCA of ancient southern Africans and a global comparative data set. (C) Admixture analysis, for selected clusters (K), fig. S8 for all K values.

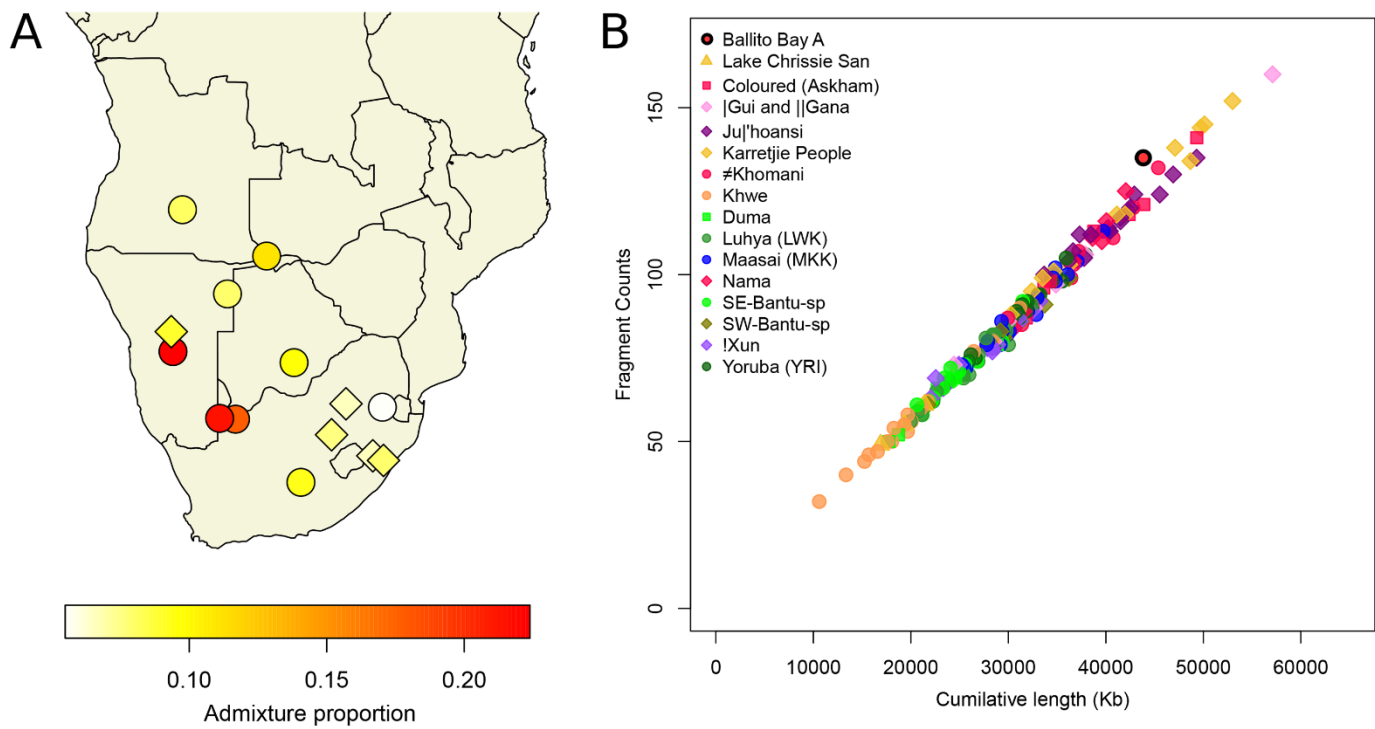


Fig. 2. (A) East African/Eurasian admixture proportions (f_4 ratio test using Amhara as the reference population) for current-day southern African populations (6). Circles depict San and Khoekhoe populations and diamonds Bantu-speakers. (B) Runs of homozygosity of the 200-500 Kb bin in Africans.

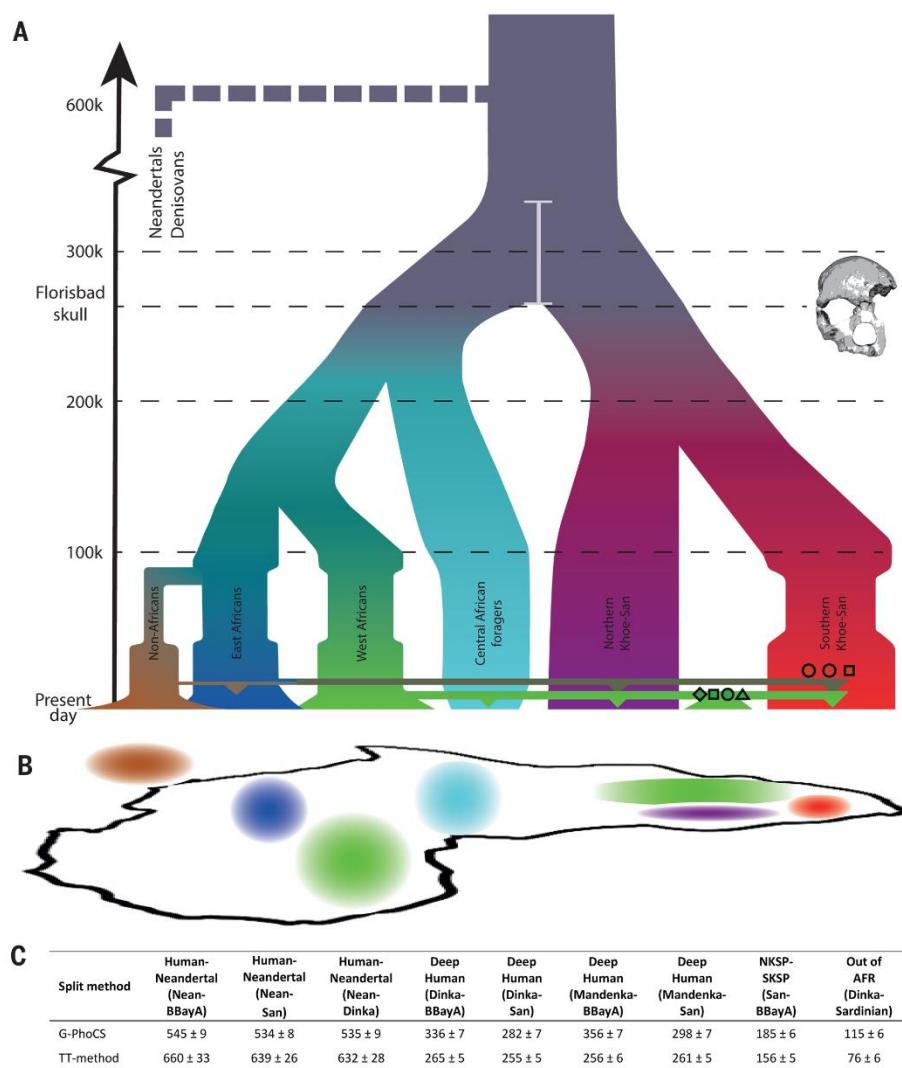


Fig. 3. Demographic model of African history and estimated divergences. (A) Population split-times, hierarchy and population sizes (width along a horizontal axis for populations) (summary of results in figs. S6, S12, S18, S19, S20, and S24). Vertical colored lines represent migration, with down-pointing triangles representing admixture into another group. The estimated age of the Florisbad skull (24) is indicated by a dashed line. 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 is the estimated split-time between Ballito Bay A and Ju|'hoansi.

Table 1. Samples [see (13)].

| Sample | Calibrated date BP (2σ) | 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 |

*Ribot *et al.* (2010) (15)

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

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