

HUMAN EVOLUTION

Denisovan ancestry and population history of early East Asians

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We present analyses of the genome of a ~34,000-year-old hominin skull cap discovered in the Salkhit Valley in northeastern Mongolia. We show that this individual was a female member of a modern human population that, following the split between East and West Eurasians, experienced substantial gene flow from West Eurasians. Both she and a 40,000-year-old individual from Tianyuan outside Beijing carried genomic segments of Denisovan ancestry. These segments derive from the same Denisovan admixture event(s) that contributed to present-day mainland Asians but are distinct from the Denisovan DNA segments in present-day Papuans and Aboriginal Australians.

Modern humans may have been present in East Asia as early as 80,000 years before the present (BP) (1, 2), but how they eventually settled in the region remains largely unknown (3–5). To date, genomic data from the first half of the Upper Paleolithic in East Asia exist only for a single human, a ~40,000-year-old individual from Tianyuan Cave in the Beijing area in China (6). This individual was more closely related to present-day East Asians than to ancient Europeans but, surprisingly, shared more alleles with a ~35,000-year-old individual from Belgium (Goyet Q116-1) than with other ancient Europeans (7). In Siberia, which neighbors East Asia to the north, four modern human individuals older than 20,000 years BP have been studied: a ~45,000-year-old individual from Ust'-Ishim in West Siberia who did not contribute ancestry to present-day populations (8); a ~24,000-year-old individual from Mal'ta 1 in South Central Siberia who was more related to Western Europeans than to East Asians and was part of a population that contributed approximately one-third of the ancestry of present-day Native Americans (9); and two ~31,000-year-old individuals from the Yana Rhinoceros Horn Site in northeastern Siberia who show affinities to early modern humans in both West and East Eurasia (10).

In 2006, a hominin skull cap was discovered during mining operations in the Salkhit Valley in northeastern Mongolia (48°16'17.9" N, 112°21'37.9" E) (11) (Fig. 1A). Its unusual morphology led to it being referred to as

Mongolanthropus (11) and later to suggestions that it was affiliated with Neanderthals or *Homo erectus* (12–14). Recently, it was radiocarbon-dated to 33,900 to 34,950 calibrated years BP (95% probability interval), and its mitochondrial (mt) DNA was shown to belong to a basal lineage of the N-haplogroup of modern human mtDNAs that are widespread in Eurasia today (15).

To study the Salkhit individual's nuclear genome, we generated shotgun sequence data from six DNA libraries prepared from bone powder sampled from the Salkhit skull cap (fig. S1) (16). Between 0.6% and 5.6% of the DNA fragments in the libraries mapped uniquely to the human reference genome (hg19) (table S2). Apparent cytosine (C) to thymine (T) substitutions, which are common at the ends of ancient DNA molecules as a result of deamination of cytosine residues (17), affect 23% to 40% of the 5'-ends and 13% to 25% of the 3'-ends in the six libraries, indicating the presence of ancient hominin DNA (table S3 and figs. S2 and S3). Using C to T substitution patterns (18), we estimate the extent of contamination by present-day human DNA to vary between ~5% and ~50% among the libraries (table S3). Because of the high level of human contamination, subsequent analyses (unless specified otherwise) were performed using only DNA sequences showing evidence of cytosine deamination at their first or last base (referred to as “deaminated fragments”), among which contamination is estimated at 1 to 3% (table S4).

The average coverage of the autosomes is similar to that of the X chromosome, indicating that despite the robust morphology of the skull, the Salkhit individual was female (table S8 and fig. S7). To determine to which major group of hominins she belonged, we estimated the percentage of derived alleles shared with the genomes of a present-day human (Mbuti, HGDP00982) (19), a Neanderthal (Denisova 5) (20), and a Denisovan (Denisova 3) (21). We found that 32% of informative posi-

tions covered by deaminated fragments carry alleles seen in the present-day human, whereas 5% and 7% carry alleles seen in the Neanderthal and Denisovan genomes, respectively. This falls within the range seen for present-day Eurasian individuals (table S9 and fig. S8), indicating that the Salkhit individual was a modern human, in agreement with more recent morphological analyses (13, 14).

To investigate the relationship of the Salkhit individual to ancient and present-day modern humans, we enriched the libraries for human DNA fragments by hybridization capture using oligonucleotide probes targeting ~2.2 million single-nucleotide polymorphisms (SNPs) selected to be informative about modern human population history (22–24). Of these, 28% were covered by deaminated DNA fragments in the Salkhit libraries.

We inferred the extent of genetic similarity [using “outgroup” *f*₃ statistics and *D* statistics (25)] between the Salkhit individual, modern human individuals older than 20,000 years (table S1), and 131 present-day populations (19). The Salkhit individual, similar to the ~40,000-year-old Tianyuan individual from China, is more related to present-day East Eurasians and Native Americans than to West Eurasians (Fig. 1B, table S10, and fig. S10). Both early East Asians are equally related to most present-day East Eurasians and Native Americans (Fig. 1C and table S12) but differ in their affinity to West Eurasians; present-day West Eurasians share more alleles with the Salkhit individual than with the Tianyuan individual (Fig. 1C). Additionally, the Salkhit individual shares as many alleles with the Tianyuan individual as with the ~31,000-year-old Yana individuals from northeastern Siberia (tables S12 and S14), yet the Tianyuan and Yana individuals share fewer alleles with each other than with the Salkhit individual (Fig. 1C and tables S12 to S14). Those observations suggest that gene flow occurred between populations ancestral to the Salkhit individual and the Yana individuals before ~34,000 years BP—that is, between early populations in East Asia and in Siberia following the divergence of East and West Eurasians. The ~35,000-year-old Goyet Q116-1 individual from Belgium shares more alleles with the Salkhit and Tianyuan individuals (7) than do other Europeans analyzed to date (tables S15 and S16). The fact that the Salkhit individual shares even more alleles with the Goyet Q116-1 individual than does the Tianyuan individual (Fig. 1C) is probably due to gene flow bringing West Eurasian ancestry into the ancestors of the Salkhit individual.

Population admixture models that are compatible with genomic data from modern human individuals older than 20,000 years were evaluated using qpGraph (25) (Fig. 2 and fig. S14). Our models suggest that the Tianyuan individual and the ~37,000-year-old

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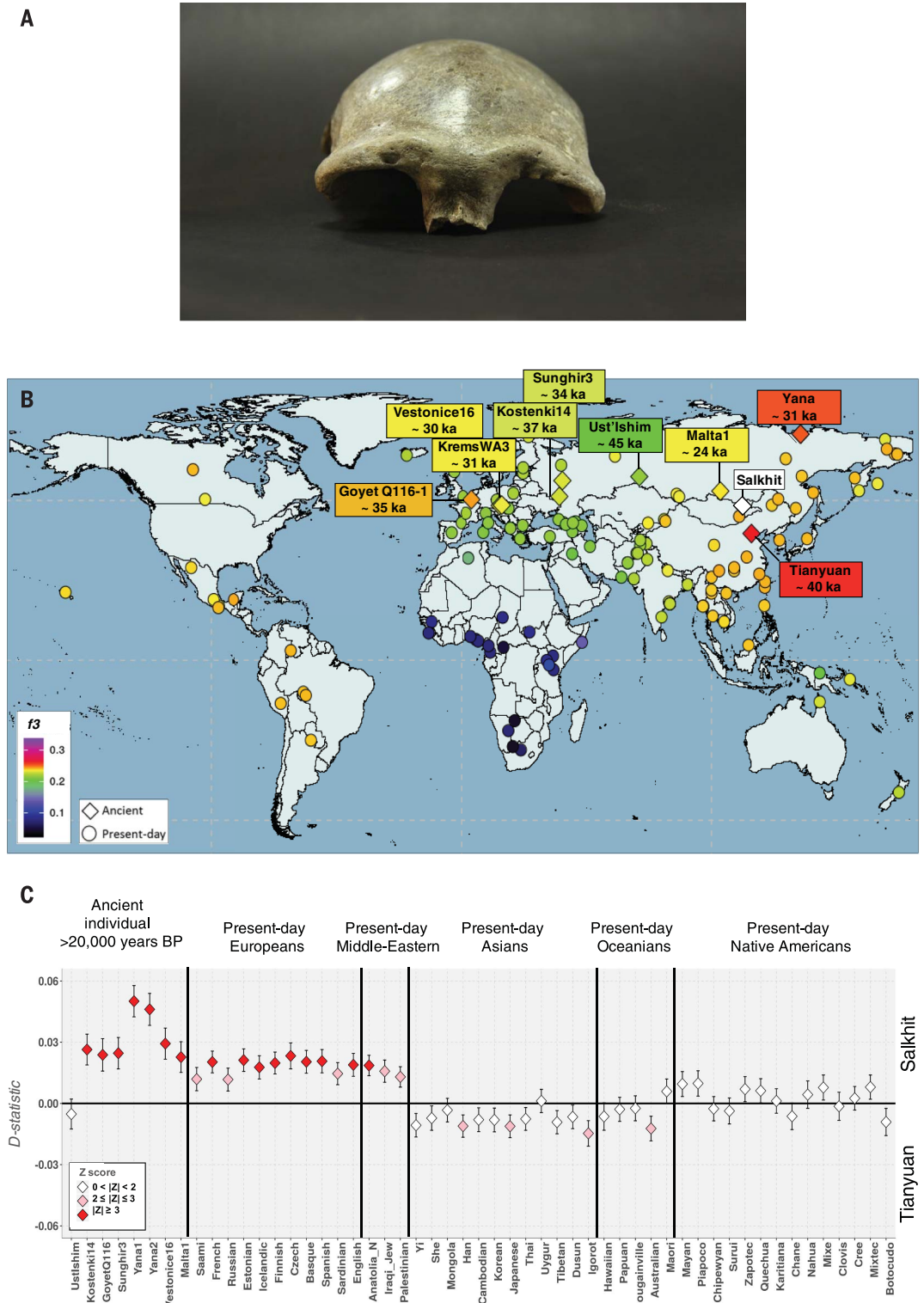
Kostenki14 individual from Russia are unadmixed representatives of early East and West Eurasian populations, respectively. The Salkhit individual, who lived in Mongolia about 6000 years after the Tianyuan individual, carries ~75% of its ancestry from a Tianyuan-related East Eurasian population and the re-

maining ~25% from a population related to the Siberian Yana individuals, who lived some 3000 years later than the Salkhit individual. In agreement with previous results (10), the Yana individuals are estimated to have about one-third of their ancestry from early East Eurasians and the remaining two-thirds from the early

West Eurasians. Their relationship to the Salkhit individual is complex: Models without bidirectional gene flow between an East Asian population ancestral to the Salkhit and a population related to the Yana individuals do not fit the data (fig. S14). Thus, some time before 34,000 years ago, gene flow from West to East Eurasia

Fig. 1. The Salkhit individual and her relationship to ancient and present-day humans.

(A) The Salkhit skull cap. [Image © Institute of Archaeology, Mongolian Academy of Sciences (Mongolia)] **(B)** Heat map illustrating the genetic similarity between the Salkhit individual and modern humans from Eurasia older than 20,000 years (20 ka) (diamonds) as well as present-day human populations (circles) determined by f_3 statistic of the form $f_3(\text{Salkhit}, X; \text{Mbuti})$. The warmer the color, the higher the genetic similarity between the Salkhit individual and a population or individual. **(C)** Relative amounts of allele sharing between the Salkhit and Tianyuan genomes and ancient and present-day humans determined by D statistics of the form $D(\text{Salkhit}, \text{Tianyuan}, X, \text{Mbuti})$. The D statistic is positive when the individual/population shares more alleles with the Salkhit individual than with the Tianyuan individual. The colors of the diamonds indicate whether the Z-score is significant (red), weakly significant (pink), or not significant (white).



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occurred, probably mediated by ancestors of the early colonizers of Siberia as represented by the Yana individuals.

We estimate the proportion of Neanderthal ancestry in the Salkhit genome to be ~1.7% (table S17 and fig. S15), similar to other early Eurasians.

As is the case for other Eurasian individuals, the Neanderthal ancestry in the Salkhit individual is equally related to the two Neanderthals from Vindija Cave in Croatia and Chagyrskaya Cave in Siberia who are ~50,000 and 80,000 years old, respectively, and less related to the ~120,000-year-

old Siberian “Altai” Neanderthal from Denisova Cave (20, 26, 27) (fig. S16).

In addition to the Neanderthal ancestry, present-day individuals in East Asia carry ancestry from Denisovans, although in mainland Asia the amount of Denisovan ancestry in present-day populations is less than the amount of Neanderthal ancestry by a factor of 10 (28–30). This has hitherto made it impossible to determine whether ancient genomes from Asia, which are of lower quality than present-day genomes, carry Denisovan ancestry. We applied a novel hidden Markov approach (31) that is able to identify introgressed Neanderthal and Denisovan genomic segments in low-coverage ancient genomes; this method uses a genotype likelihood approach that incorporates contamination, so that all fragments can be used for this analysis. Using data from ~1.7 million SNPs where Neanderthal and/or Denisovan genomes differ from present-day African genomes, we detected 18 segments of Denisovan ancestry longer than 0.2 cM in the Salkhit genome (Fig. 3, table S18, and figs. S17 and S28) and 20 such segments in the Tianyuan genome (table S18 and figs. S19 and S28). We detected about one-third as many

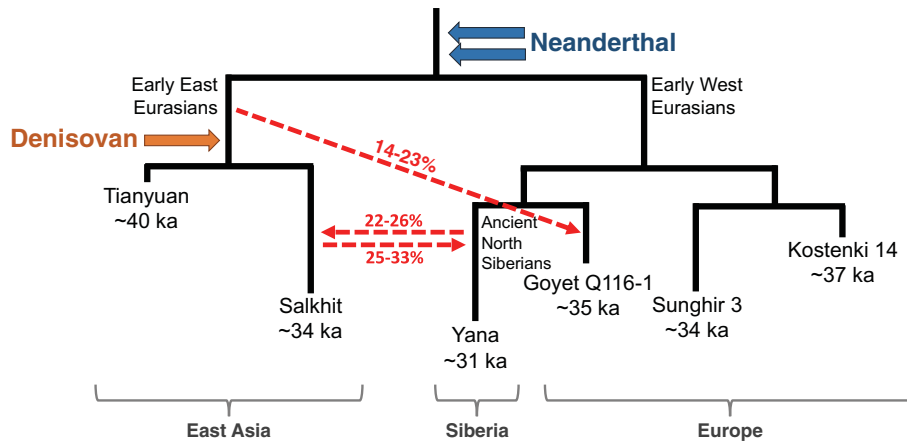
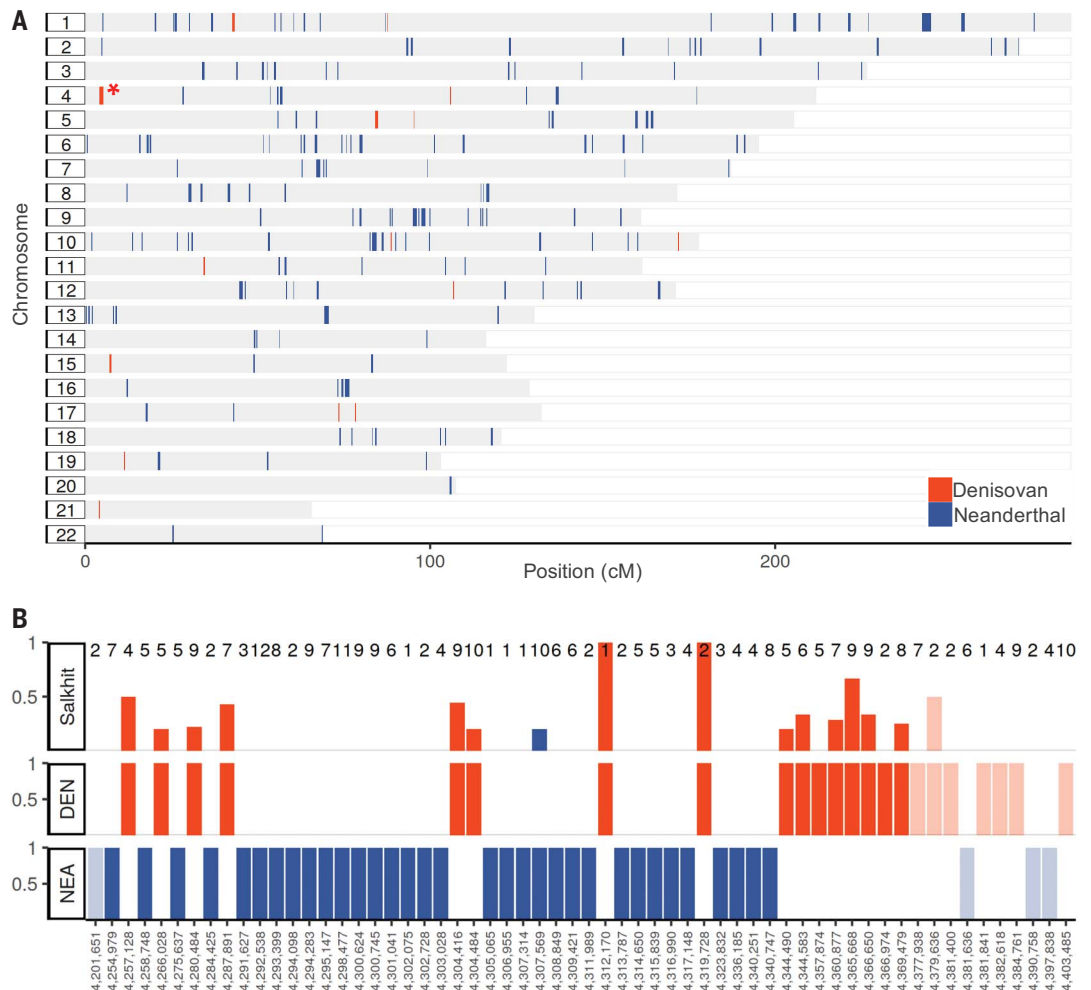


Fig. 2. Simplified demographic model including the Salkhit individual and other Eurasians older than 30,000 years. Admixtures between East and West Eurasians are represented by red arrows. Neanderthal and Denisovan admixtures are indicated by blue and orange arrows, respectively.

Fig. 3. Archaic ancestry in the Salkhit genome.

(A) Genomic distribution of Denisovan (orange) and Neanderthal (blue) DNA segments in the Salkhit genome. (B) Allele frequencies in the longest Denisovan ancestry segment [chr4: 4.16 to 5.31 cM, marked by an asterisk in (A)]. The bars in the top panel give the proportions of Salkhit DNA fragments carrying archaic alleles at sites where alleles are fixed between Africans and the Denisovan genome (red) or between Africans and two Neanderthal genomes (blue). Total numbers of fragments are shown at the top. SNP bars outside the inferred Denisovan segment are faded. Note that in the called region, all Denisovan-like alleles except two occur in the Salkhit genome.



segments of Denisovan DNA in the genomes of the ancient Siberians Yana 1, Yana 2, and Mal'ta 1 (table S18, figs. S19 to S21, and fig. S28), consistent with the proportion of their East Asian ancestry. In contrast, no Denisovan ancestry was detected in the genome of the ~45,000-year-old Siberian individual from Ust'Ishim in West Siberia, nor in any European individual older than 20,000 years (table S18, figs. S22 to S25, and fig. S28). Thus, the Salkhit and Tianyuan genomes provide direct evidence that ancestors of modern humans who lived in

East Asia 40,000 years ago had met and mixed with Denisovans. The small number of these segments does not provide enough power to date the introgression event. However, given their relatively short length (≤ 1.3 cM), the Denisovan introgression is likely to have happened at least 10,000 years before these individuals lived.

One of the risks of inferring ancestry fragments from ancient genomes is that genome quality may affect the ability to detect introgressed segments. Under the assumption that

many of the factors that affect the detection of Denisovan DNA will similarly affect the detection of Neanderthal DNA, the ratio of Denisovan to Neanderthal ancestry segments may be a reasonably robust metric of the relative amount of Denisovan ancestry. In the Salkhit and Tianyuan genomes, these ratios are about 7.5% and 8.1%, respectively. For the genomes of the North Siberians Yana 1 and Yana 2, the ratios are about 3.9% and 4.7%. Because there is no substantial difference in the amounts of Neanderthal DNA in the two

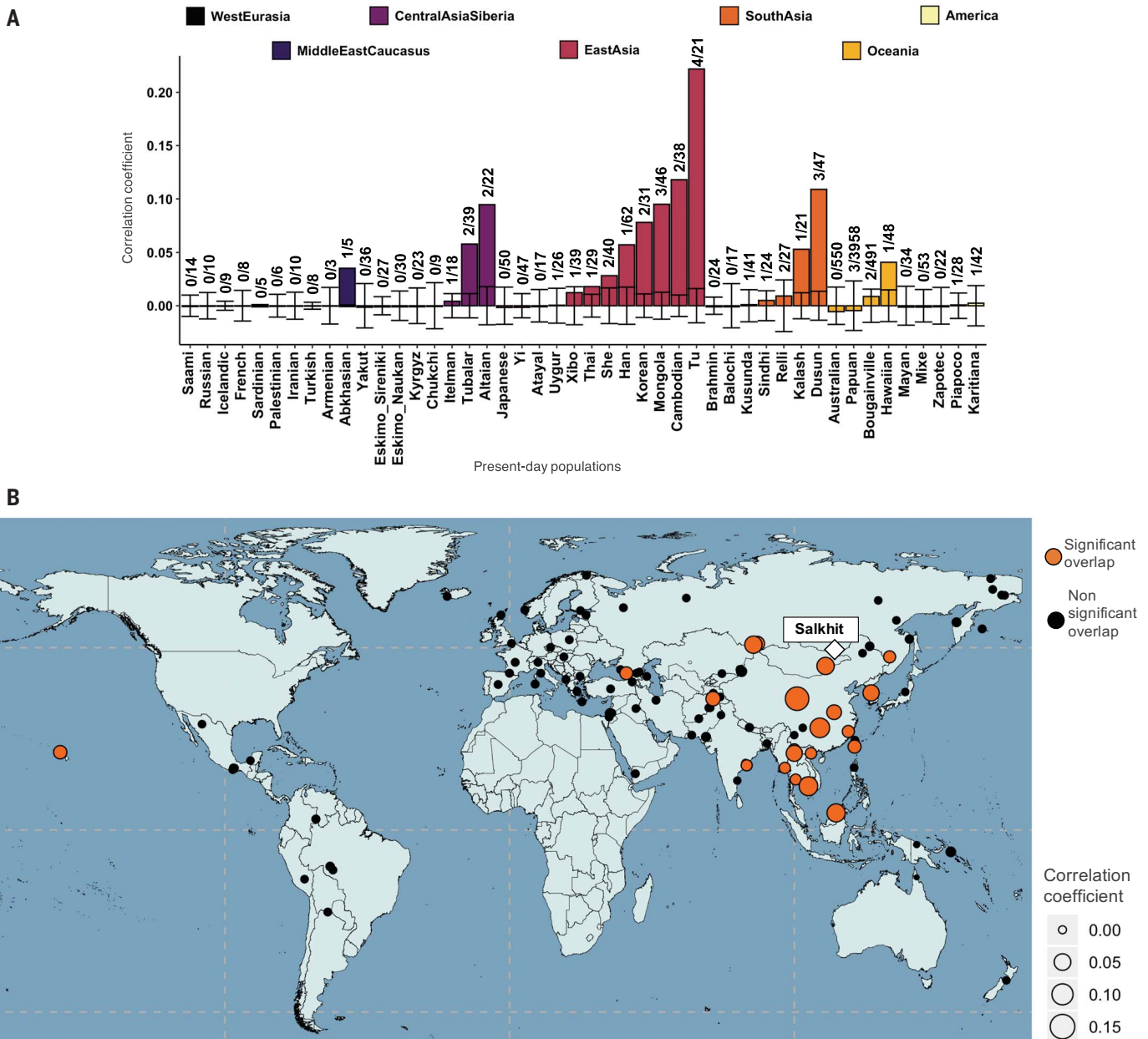


Fig. 4. Overlap of Denisovan segments in the Salkhit genome and present-day non-African populations. (A) Correlation coefficient of the overlap between the Denisovan segments larger than 0.2 cM in the Salkhit genome and Denisovan segments larger than 0.05 cM in 45 present-day Eurasian populations (see fig. S28 for the same with 111 present-day populations). Numbers above the bars

give the number of overlapping segments and the number of segments in the present-day population. The range of correlation coefficients generated by 500 bootstraps is indicated. **(B)** Geographic locations of present-day populations for which Denisovan ancestry segments overlap significantly with the Salkhit individual (orange circles). Sizes of circles are proportional to the correlation coefficients.

early East Asian genomes and the two Yana genomes (fig. S15), this observation indicates that the ancient Siberian individuals carry less Denisovan DNA than the Salkhit and Tianyuan individuals.

We compared the Denisovan segments in the Salkhit and Tianyuan genomes to those in present-day people to estimate whether introgressed segments between genomes overlap more often than expected by chance. Significance was assessed using 500 bootstrap reshuffles, where segments are randomly relocated across the analyzed genomes (supplementary text). The Denisovan DNA segments in the ancient East Asian genomes overlap more than expected with Denisovan segments detected in the genomes of several present-day populations in Asia and in populations with some Asian ancestry, such as Hawaiians (Fig. 4, table S19, and figs. S29 to S31). In contrast, we find no significant overlap with Denisovan segments detected in Papuans or Aboriginal Australians, although these groups carry on the order of 20 times as much Denisovan DNA as mainland Asians.

It has been shown that at least two Denisovan populations contributed ancestry to present-day East Asian populations, and that Denisovan ancestry in populations in Oceania derived from only one of these sources (29). The overlap of Denisovan DNA segments (Fig. 4, table S19, and figs. S29 to S31) is in agreement with this and suggests that the ancestral population of the Tianyuan and Salkhit individuals that mixed with Denisovans contributed ancestry to populations in large parts of Asia today. In contrast, the lack of any significant overlap with Aboriginal Australians and Papuans suggests that these Oceanian populations received most of their Denisovan ancestry from a different source.

Our results show that the 34,000-year-old Salkhit individual carried more West Eurasian ancestry than the 40,000-year-old Tianyuan individual, indicating that after the major West/East Eurasia split, gene flow from West Eurasia to East Asia occurred earlier than 34,000 years ago, probably mediated by populations related to the Siberian Yana individuals. We also show that these early East Asians carried segments of Denisovan DNA that come from admixture events that also contributed Denisovan DNA to populations across mainland Asia today, but not to Papuans and Aboriginal Australians.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S31
Tables S1 to S19
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MDAR Reproducibility Checklist

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DNA analyses of an early East Asian

Ancient, anatomically modern humans interbred with the archaic hominins Neanderthals and Denisovans. However, the extent of this interbreeding and how it affects modern populations is not well understood. Massilani *et al.* generated genome-wide data from a 34,000-year-old female individual from the Salkhit Valley in eastern Mongolia and conducted a detailed modeling of her ancestry with regard to other Pleistocene human genomes. They found evidence for Denisovan ancestry in ancient human genomes from at least 6000 years before the Salkhit individual lived and determined that the Denisovan contribution differed from that of another ancient Asian individual, as well as from the ancient Denisovan contribution to extant Australasians. This reference point helps us to understand the early history of our species in Eurasia, especially Eastern Eurasia, for which genomic evidence remains scarce.

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