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Figs. S1 and S2

References

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Ancient Biomolecules from Deep Ice Cores Reveal a Forested Southern Greenland

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It is difficult to obtain fossil data from the 10% of Earth's terrestrial surface that is covered by thick glaciers and ice sheets, and hence, knowledge of the paleoenvironments of these regions has remained limited. We show that DNA and amino acids from buried organisms can be recovered from the basal sections of deep ice cores, enabling reconstructions of past flora and fauna. We show that high-altitude southern Greenland, currently lying below more than 2 kilometers of ice, was inhabited by a diverse array of conifer trees and insects within the past million years. The results provide direct evidence in support of a forested southern Greenland and suggest that many deep ice cores may contain genetic records of paleoenvironments in their basal sections.

The environmental histories of high-latitude regions such as Greenland and Antarctica are poorly understood because much of the fossil evidence is hidden below kilometer-thick ice sheets (1–3). We test the idea that the basal sections of deep ice cores can act as archives for ancient biomolecules.

The samples studied come from the basal impurity-rich (silty) ice sections of the 2-km-long Dye 3 core from south-central Greenland (4), the 3-km-long Greenland Ice Core Project (GRIP) core from the summit of the Greenland ice sheet (5), and the Late Holocene John Evans Glacier on Ellesmere Island, Nunavut, northern Canada (Fig. 1). The last-mentioned sample was included as a control to test for potential exotic DNA because the glacier has recently overridden a land surface with a known vegetation cover (6). As an additional test for long-distance atmospheric dispersal of DNA, we included five control samples of debris-free Holocene and Pleistocene ice taken just above the basal silty samples from the Dye 3 and GRIP ice cores (Fig. 1B). Finally, our analyses included sediment samples from the Kap København Formation from the northernmost part of Greenland, dated to 2.4 million years before the present (Ma yr B.P.) (1, 2).

The silty ice yielded only a few pollen grains and no macrofossils (7). However, the Dye 3 and John Evans Glacier silty ice samples showed low levels of amino acid racemization (Fig. 1A, inset), indicating good organic matter preservation (8). Therefore, after previous success with permafrost and cave sediments (9–11), we attempted to amplify ancient DNA from the ice. This was done following strict criteria to secure authenticity (12–14), including covering the sur-

face of the frozen cores with plasmid DNA to control for potential contamination that may have entered the interior of the samples through cracks or during the sampling procedure (7). Polymerase chain reaction (PCR) products of the plasmid DNA were obtained only from extracts of the outer ice scrapings but not from the interior, confirming that sample contamination had not penetrated the cores.

Using PCR, we could reproducibly amplify short amplicons [59 to 120 base pairs (bp)] of the chloroplast DNA (cpDNA) *rbcL* gene and *trnL* intron from ~50 g of the interior ice melts from the Dye 3 and the John Evans Glacier silty samples. From Dye 3, we also obtained 97-bp amplicons of invertebrate cytochrome oxidase subunit I (COI) mitochondrial DNA (mtDNA). Attempts to reproducibly amplify DNA from the GRIP silty ice and from the Kap København Formation sediments were not successful. These results are consistent with the amino acid racemization data demonstrating superior preservation of biomolecules in the Dye 3 and John Evans Glacier silty samples, which is likely because these samples are colder (Dye 3) or younger (John Evans Glacier) than the GRIP sample (Fig. 1A, inset). We also failed to amplify DNA from the five control samples of Holocene and Pleistocene ice taken just above the silty samples from the Dye 3 and GRIP ice cores (volumes: 100 g to 4 kg; Fig. 1B) (7). None of the samples studied yielded putative sequences of vertebrate mtDNA.

A previous study has shown that simple comparisons of short DNA sequences to GenBank sequences by means of the Basic Local Alignment Search Tool (BLAST) make misidentification likely (15). Therefore, we assigned the obtained sequences to the taxonomic levels of order, family, or genus using a new rigorous statistical approach (7). In brief, this Bayesian method calculates the probability that each sequence belongs to a particular clade by considering its position in a phylogenetic tree based on similar GenBank sequences. In the calculation of these probabilities, uncertainties regarding phylogeny, models of evolution, and missing data are taken into account. Sequences with >90% posterior probability of membership to a taxonomic group were assigned to that group. Additionally, a given plant taxon was only considered genuine if sequences assigned to that taxon were found to be reproducibly obtained in separate analyses (by independent laboratories for the Dye 3 sample and within the laboratory for the John Evans Glacier control sample). This strict

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herbaceous plants and are represented by many species found in northern regions at present (Table 1). The presence of these herb-dominated families suggests an open forest where heliophytes thrived. Additionally, we recorded taxa that are common in present-day Arctic and/or boreal regions but lacked identity between independent laboratories. These are yarrow (*Achillea*), birch (*Betula*), chickweed (*Cerastium*), fescue (*Festuca*), rush (*Luzula*), plantain (*Plantago*), bluegrass (*Poa*), saxifrage (*Saxifraga*), snowberry (*Symphoricarpos*), and aspen (*Populus*). Although not independently authenticated at the sequence level, the presence of these taxa adds further support to the former existence of a northern boreal forest ecosystem at Dye 3.

To date, the youngest well-dated fossil evidence of native forest in Greenland is from macrofossils in the deposits of the Kap København Formation from the northernmost part of Greenland and dates back to around 2.4 Ma (1, 2). Other less well dated traces of forests in Greenland include wood at two other late Cenozoic sites in northern Greenland (20), pollen spectra of unknown age in marl concretions found in a late glacial moraine, and wood and spruce seeds in eastern Greenland (21). The core from Dye 3, located almost exactly 2000 km to the southwest of the Kap København Formation (Fig. 1A), therefore, provides direct evidence of a forested southern-central Greenland.

The invertebrate sequences obtained from the Dye 3 silty ice are related to beetles (Coleoptera), flies (Diptera), spiders (Arachnida), brush-foots (Nymphalidae), and other butterflies and

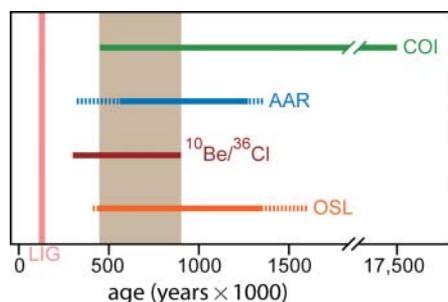


Fig. 2. Summary of dating results for the silty ice from Dye 3. From top to bottom, the bars indicate: maximum likelihood estimates for the branch length of the invertebrate COI sequences (COI); amino acid racemization results with the use of alternative activation energies, models of racemization behavior, and basal temperature histories (AAR); age estimate from $^{10}\text{Be}/^{36}\text{Cl}$ measurements in silty ice; and minimum ages based on single-grain luminescence results (optically stimulated luminescence or OSL). The time span covered by all dating methods (450 to 800 ka) is marked in gray. Stippled lines represent the results of less likely models. The maximum age estimate for the invertebrate COI sequences is based on an unlikely slow substitution rate [for details, see text and (6)].

moths (Lepidoptera) (taxonomic identification probability support between 50 and 99%). However, only sequences of the Lepidoptera are supported by more than 90% significance (Table 1). Thus, although detailed identifications of the COI sequences are in general not strongly supported, the results show that DNA from a variety of invertebrates can be obtained from sediments even in the absence of macrofossils, as was previously shown for plants, mammals, and birds (9–11).

Several observations suggest that the DNA sequences we obtained from the Dye 3 ice are of local origin and not the result of long-distance dispersal. The reproducible retrieval of diverse DNA from the silty basal ice but not from similar or larger volumes of the overlying “clean” ice largely precludes long-distance atmospheric dispersal of microfossils as a source of the DNA.

Although pollen grains are found in the Greenland ice sheet, including the Dye 3 silty ice (7), the concentrations are in general too low [~15 grains per liter (22, 23)] for them to be present in the sample volumes studied. Furthermore, long-term survival of DNA in pollen has proved fairly poor (24), and the vast majority of angiosperm pollen does not contain cpDNA (25). These factors effectively exclude pollen as the general source of plant DNA from the silty ice. Moreover, the Dye 3 silty ice appears to have originated as solid precipitation without going through stages of superimposed ice and most likely formed by mixing in the absence of free water (i.e., ice that has never melted) (26), effectively excluding subsurface transportation. As explained in (27), the ice is believed to be predominantly of local origin, having been shielded from participating in the large-scale glacier flow by a bedrock trough, in agreement with the solid ice–mixing hypothesis (26). Thus, being of local origin, the DNA sequences from the Dye 3 silty ice must be derived from the plants and animals that inhabited this region the last time that it was ice-free, because possible older DNA records from previous ice-free periods will vanish with the establishment of a new ecosystem, or at least be out-competed during PCR by DNA from the most recent record. The plant taxa suggest that this period had average July temperatures that exceeded 10°C and winter temperatures not colder than -17°C , which are the limits for northern boreal forest and *Taxus*, respectively (1). Allowing for full recovery of the isostatic depression that is produced by 2 km of ice, Dye 3 would have been ~1 km above sea level. In combination, these factors suggest that a high-altitude boreal forest at Dye 3 may date back to a period considerably warmer than present.

There are no established methods for dating basal ice, and it remains uncertain whether the overlying clean ice of Dye 3 is temporally contiguous with the lower silty section. Therefore, to obtain a tentative age estimate for the Dye 3

silty ice and its forest community, we applied a series of dating techniques: $^{10}\text{Be}/^{36}\text{Cl}$ isotope ratios, single-grain luminescence measurements, amino acid racemization coupled with modeling of the basal ice temperature histories of GRIP and Dye 3, and maximum likelihood estimates for the branch length of the invertebrate COI sequences (7). All four dating methods suggest that the Dye 3 silty ice and its forest community predate the Last Interglacial (LIG) [~130 to 116 thousand years ago (ka)] (Fig. 2), which contrasts with the results of recent models suggesting that Dye 3 was ice-free during this period (28, 29). Indeed, all four dating methods give overlapping dates for the silty ice between 450 and 800 ka (Fig. 2), exceeding the current record of long-term DNA survival from Siberian permafrost of 300 to 400 ka (9). However, because of the many assumptions and uncertainties connected with the interpretation of the age estimates (7), we cannot rule out the possibility of a LIG age for the Dye 3 basal ice.

Our results reveal that ancient biomolecules from basal ice offer a means for environmental reconstruction from ice-covered areas and can yield insights into the climate and the ecology of communities from the distant past. Because many deep ice cores exist from both hemispheres and further drillings are planned, this approach may be used on a larger scale. Basal ice at even lower temperatures than Dye 3 might contain an archive of genetic data of even greater antiquity.

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Middle Paleolithic Assemblages from the Indian Subcontinent Before and After the Toba Super-Eruption

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The Youngest Toba Tuff (YTT) eruption, which occurred in Indonesia 74,000 years ago, is one of Earth's largest known volcanic events. The effect of the YTT eruption on existing populations of humans, and accordingly on the course of human evolution, is debated. Here we associate the YTT with archaeological assemblages at Jwalapuram, in the Jurreru River valley of southern India. Broad continuity of Middle Paleolithic technology across the YTT event suggests that hominins persisted regionally across this major eruptive event.

The Youngest Toba Tuff (YTT) eruption of 74,000 years ago (74 ka) was Earth's largest volcanic event in the past two million years (1–3). It was two orders of magnitude larger (in erupted mass) than the largest known historic eruption, that of Tambora, also in Indonesia (4). The YTT involved the eruption of a minimum of 2800 km³ (7×10^{15} kg) of magma, of which at least ~800 km³ was transported in atmospheric ash plumes that blanketed an area from the South China Sea to the Arabian Sea (2, 3). Its impact on Earth's atmosphere and climate (5–7) and on local animal and plant populations remains a matter of contention (5, 7–12).

The Indian subcontinent contains extensive YTT deposits (13–15). Here we describe an archaeological sequence from south India that includes a substantial YTT layer and sheds light on the eruption's impact on climate, environments, and hominin populations. In the Kurnool District

of Andhra Pradesh in southern India, stratified archaeological sites in the Jurreru River valley contain stone artifacts in association with faunal remains in caves, rockshelters, and open-air localities (16, 17) (Fig. 1). The archaeological record spans all periods of the Paleolithic. In addition, current mining activities have exposed tephra deposits over an area of 64 ha. Ash is, however, certainly buried over a wider area within the valley (fig. S1), and we estimate its total volume at $7 \pm 0.7 \times 10^5$ m³, based on the interpolation of 225 depth observations made at mining exposures.

We conducted electron probe microanalysis (EPMA) of volcanic glass shards from the Jwalapuram tephra to compare their geochemical signatures with those of the Older Toba Tuff (OTT, dated to ~840 ka) and the Middle Toba Tuff (MTT, dated to ~500 ka) (4). The results show that the Jwalapuram ash is a distal deposit of the YTT (figs. S3 and S4), based on its close similarities with proximal deposits of YTT in Sumatra and with previously characterized distal occurrences in India (13, 14, 18).

Jwalapuram locality 3 preserves more than 7.5 m of sedimentary deposits, including a 2.55-m-thick deposit of ash, and a sequence of lithic artifacts that straddle the ash layer (fig. S2). Soft sediment deformation structures suggest that the tephra initially accumulated on a wet clay substrate, probably in a lacustrine environment. The abrupt transition from light gray ash to an orange (but still ash-rich) silt horizon immediately above the ash sequence represents a major change in depositional regime. We interpret this as evidence that the lake dried up soon after the ash fall, possibly during the onset of glacial conditions in oxygen isotope stage 4.

The stone tool assemblages were found in trenches placed across the landscape (that is, at Jwalapuram localities 3, 17, and 21). At Jwalapuram locality 3, we used optical dating to obtain burial ages for sediment samples from archaeological layers above (JLP-380) and below (JLP3A-200) the ash. Ages of 77 ± 6 and $74 \pm$

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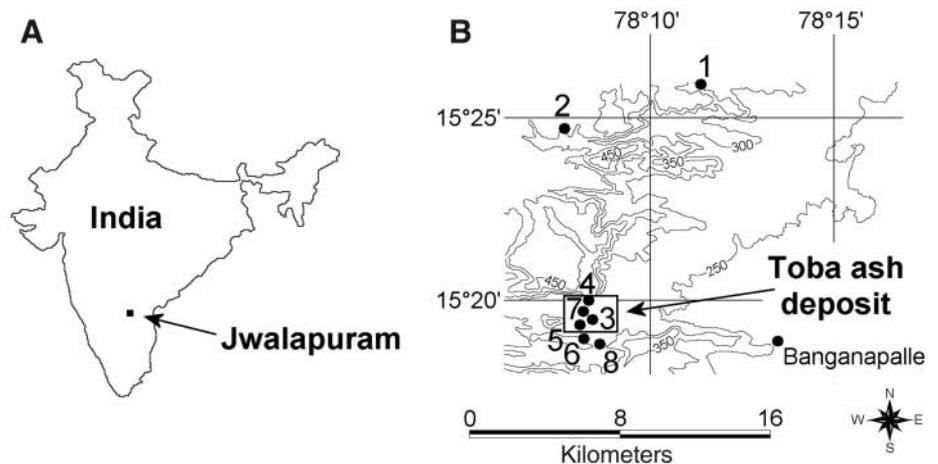


Fig. 1. Location of Jwalapuram, archaeological sites, and tephra deposits. (A) Location of the Jwalapuram study area. (B) Key archaeological localities in the Kurnool District include the Upper Paleolithic caves of Billasurgum (1) (17) and Muchchatla Chintamanu Gavi (2) (16). Jwalapuram localities include 17 (3, Middle Paleolithic), 9 (4, Microlithic), 3 (5, Middle Paleolithic), 20 (6, Middle Paleolithic), 21 (7, Middle Paleolithic), and Tank (8, Acheulean).



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