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ANTHROPOLOGY

Direct evidence for psychoactive cannabis use 2800 years ago

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Cannabis is the world's most-used illicit psychoactive plant, yet direct prehistoric evidence for its consumption is rare. In this study, we present the earliest direct evidence of cannabis use: offerings of plant parts and chemical compounds in ancient human hair recovered from a monumental tomb in Siberia. The findings suggest that the plant was used for its psychoactive qualities across Eurasia, where specific wild populations might have had naturally higher active compound levels. We show that the practice of cannabis consumption dates back at least 2800 years, anchoring debates around contemporary usage and informing discussions on cannabis in medicine, public health, law, and industry.

Introduction

Cannabis (*Cannabis sativa* spp. *indica*) ranks as the third most commonly used controlled substance globally, after alcohol and tobacco (*1*). According to a 2018 United Nations estimate, ~3.9% of the global population had used cannabis within the previous year (*2*); this number increased to 4.41% in 2022 (*3*). In its modern domesticated form, the plant material, or its extracts, often contain considerable levels of tetrahydrocannabinol (THC), the active compound that binds to endocannabinoid type 1 receptors in the brain and produces a euphoric or mind-altering sensation (*4*). Psychoactive plant use is a near cultural universal, maintaining medicinal, recreational, and ritual importance in cultures around the world (*5*–*7*). Despite its widespread impact, use of this plant has rarely been recorded archaeologically; recovery rates are reduced by the ephemeral nature of plant residues. The dearth of evidence has led to speculation regarding the prehistoric use of such substances and associated deep time cultural practices because, even with surviving plant remains, direct evidence of consumption is often still lacking. There has been increasing interest in the still-unresolved questions of the origins of cannabis use and cultivation and when it evolved traits of domestication (*8*–*11*). Scholars interested in this topic have looked to Central Asia for the earliest evidence of ritual use, and archaeological evidence has already demonstrated that ancient communities were using it and possibly even selecting naturally occurring, more psychoactive varieties of the plant by 2500 years ago (*9*).

Cannabis exists in a taxonomically unresolved hybrid complex with wild populations growing in the temperate, mountainous zones stretching from the Caucasus to western China (*10*, *12*). Although they are often considered ruderal weeds associated with human disturbance, their natural habitat is frequently proposed to have been associated with riparian (*8*) or grazing disturbance (*10*). Their association with grazing disturbance and, correlatively, ruminant seed dispersal, would have translated well into pastoralist landscapes of the early first millennium BCE, whereas herd animal grazing may have concentrated wild cannabis stands (as it does today) (*10*). Cannabis was part of the natural flora across northern Central Asia, as confirmed by pollen data from both lake sediments (*13*) and soil buried under a fourth-century-BCE Scythian burial mound (*14*). However, in all cases, these are sporadic finds of cannabis pollen. Among these regions, the foothills of mountainous Central Asia have supported naturally occurring populations, which are often classified as *C. sativa* ssp. *ruderalis*. There is considerable variation in the production of secondary compounds between wild and feral populations (*10*, *15*). Additionally, the species is recognized as expressing a wide reaction norm of developmental responses. For example, it has been proposed that high-elevation or high-latitude populations may produce higher chemical defenses in response to greater solar intensity (*16*–*18*). The wild progenitor remains unknown, and current species across Eurasia commonly classified as wild may either be feral or express high rates of crop-to-wild gene flow. It is generally accepted that one lineage of cannabis broke off from wild relatives in East Asia by at least six millennia ago, where it was first cultivated as an oil-seed crop (*8*, *9*, *12*). The East Asian cannabis evolved into a distinct lineage of domesticated crops, leading to hemp and varieties still cultivated for their seeds in parts of China today. Recent reviews have addressed the archaeological narrative of cannabis and hemp cultivation and domestication in East Asia (*11*), but the question of how the psychoactive plant lineage fits into the domestication process remains an ongoing discussion.

Despite great interest in ancient cannabis use, its early psychoactive applications are still poorly understood owing to the scarcity of archaeological evidence. A handful of discoveries—including whole plants and desiccated seeds from burials in the Taklamakan (*19*, *20*) and from the excavation of a burial in the Altai Mountains (*8*, *21*)—suggest the use of the plant in mortuary practices by around 2500 years ago (Fig. 1). Much of this scholarship also claims a link between shamanic practices and funerary rites (*9*, *20*). Yet these findings alone have not clarified when or how cannabis was first used for its mind-altering effects. An early written account from Herodotus is the most cited source for psychoactive cannabis use in antiquity, referencing inhalation of fumes through burning plant material in an enclosed space (*22*). More recently, excavations at the Jirzankal Cemetery on the Pamir Plateau have exhumed wooden braziers containing heat-altered stones from tombs dated to around 500 BCE, suggesting the intentional combustion of plant material during burial rituals (*8*). Chemical residue analysis from these artifacts revealed high concentrations of psychoactive cannabinoids, implying that the cannabis burned at this site was either selectively cultivated or derived from naturally potent local populations (*8*).

The archaeological site Tunnug 1 represents one of the earliest and most important monumental burial mounds in the Uyuk Valley of southern Siberia (*23*–*27*). This type of site forms the earliest instance of the material culture that has long been referred to as "Scythian": Similar artifacts—namely, specific types of weapons, horse gear, and animal-style motifs (*28*)—spread over the Eurasian steppe in the first millennium BCE from modern day Tuva to the Black Sea region (*29*, *30*). Sediment samples processed for archaeobotanical remains from the site have led to the identification of seed deposits of cannabis near the interred individuals in the burial, totaling more than a thousand seeds. Furthermore, human hair has been recovered from the central grave chamber, allowing for direct investigation of psychoactive compounds. In this study, we argue that these seeds were intentionally deposited and that the plant was also used for mind-altering purposes at the time.

Results

Archaeological excavation and sampling

In the center of the royal mound, four wooden structures, three with single and one with multiple burials, were uncovered. Individuals 135 and 139 were buried in tree trunk coffins typical for the earliest Scythian

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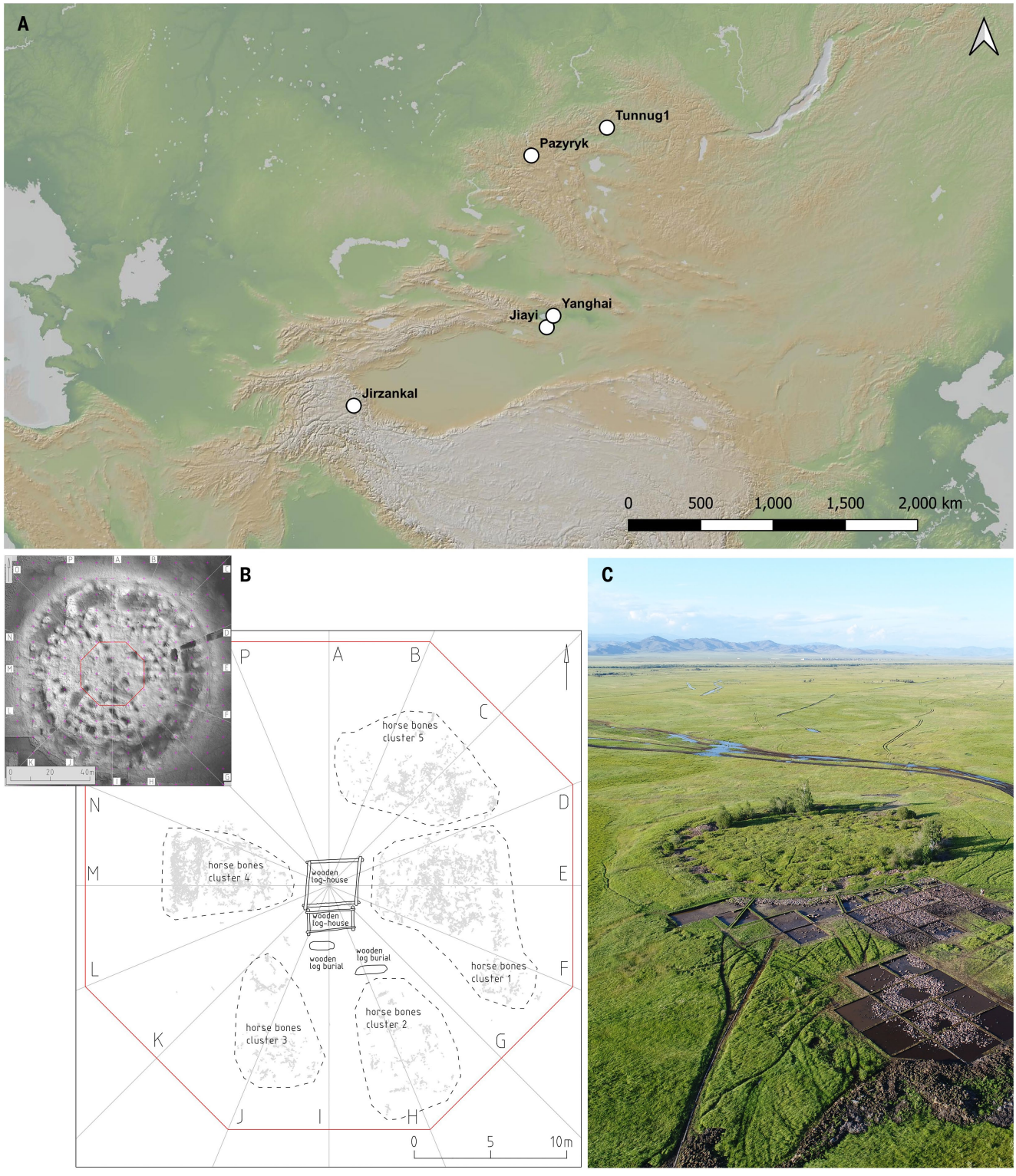


Fig. 1. Archaeological evidence for ancient cannabis use in Eurasia. (**A**) Archaeological sites with prehistoric evidence for cannabis use in Central Asia. [Illustration by G. Caspari] (**B**) The burial mound Tunnug 1 and its central part including horse sacrifices and burials. [Drawing by T. Sadykov and J. Blochin] (**C**) Oblique drone image of Tunnug 1 in the Siberian Valley of the Kings. [Photo by T. Wallace]

period in Tuva. One log cabin–like structure supported an additional log coffin for individual 140. The multiple burials contained three individuals (141 to 143), of which only 141 was buried in a log coffin. The log coffins of 135 and 139 contained seeds, as did the area around individuals 142 and 143 in the multiple burial. Both log coffins and log cabin burials were situated inside the wood-reinforced clay architecture of the burial mound (*27*), on which a large number of horses were sacrificed in the form of a spectral cavalcade (*31*). This was then covered with a layer of stones. Three sets of samples were collected during the excavations: S, including seeds that were handpicked when visually

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detected during excavation; F, including flotation samples; and H, including hair samples. The exact location of the samples is shown in Fig. 2, and their context is described in table S1.

Archaeobotanical identification

The largest accumulation of seeds was found on the eastern edge of the northern log cabin structure near individual 142. The seeds range from 2.5 to 3.5 mm in length and 1.0 to 2.0 mm in width and measure on average 2.62 mm in length, 1.97 mm in width, and 1.57 mm in thickness (see fig. S1 and table S3). They are irregularly lenticular in cross section and have a loosely pointed apex and a poorly defined hilum scar—characteristics pointing to a wild morphotype. However, some domesticated forms cultivated for their chemical compounds show little divergence from their wild relatives (*11*). The *C. sativa* spp. *ruderalis* seeds exhibit the diagnostic dendric or vascular patterning radiating from the hilum notch (Fig. 2). Wild plants and many of the morphotypes cultivated for drug use express smaller overall plant mass and correlatively smaller seeds (*8*, *11*). Dal Martello *et al.* (*11*) present the ranges of 2.01 to 3.06 mm in length and 1.35 to 2.52 mm in width for wild and feral morphs. Some scholars have also pointed out that the hilum-abscission zone on the larger-seeded forms tends to be more pronounced than on the small-seeded and the wild and feral forms (*8*). Following these criteria, the seeds from Tunnug 1 fall within the morphological range of wild forms.

We recovered a total of 722 complete cannabis seeds and 516 fragmented cannabis seeds from the flotation samples, in addition to handpicking 243 whole cannabis seeds during excavation (tables S1 and S2). Although the archaeobotanical assemblage was almost completely composed of cannabis seeds, a few additional wild seed types were recovered. These seeds may represent ancient intrusions or seed rain, possibly being inadvertently collected with cannabis plants.

Radiocarbon dating

We dated six seeds from the burial complex (Table 1), two seeds from the deposits in the northern log cabin (sample S08), two seeds from the log coffin with skeleton 139 (sample S02), and two seeds from the log coffin with skeleton 135 (sample S01). The dates coincide with a steep part of the calibration curve before the Hallstatt Plateau and, therefore, yielded a relatively narrow date range.

We created a Bayesian model, which combines the dates to further narrow the range for time of the burial (fig. S2). Sample S08 (78296 and 78295) provides the largest range because seed 78295 is an outlier and touches the Hallstatt Plateau on the calibration curve. Seed 78295 was thus excluded from the model. According to the dates of the seeds, other previously published dates (*31*, *32*), and stratigraphic information, this model further reduced the date range of the seeds, which were likely deposited sometime in the first decade of the eighth century BCE, coinciding with the dates of construction for the funerary complex.

Mass spectrometric analysis

For the mass spectrometric analysis of hair samples, partial amounts (~50 mg) were extracted with toluene. After centrifugation, evaporation, and redissolution in methanol, the extracts were separated by reversed-phase chromatography and high-resolution mass spectrometry (liquid chromatography–electrospray ionization quadrupole time-of-flight tandem mass spectrometry, LC-ESI-QTOF-MS/MS). Distinct signals matching cannabinol (CBN) were observed during analysis of all hair samples, whereas signals matching THC were comparatively weak (Fig. 3). This is plausibly a result of CBN being a refractory oxidation product of THC (*8*). Qualitative and quantitative assessments of CBN were achieved with a certified reference standard (1.0 mg/ml CBN in methanol; Cerilliant, Merck, Germany). Because no reference standard of THC was readily available, for legislative reasons, the substance

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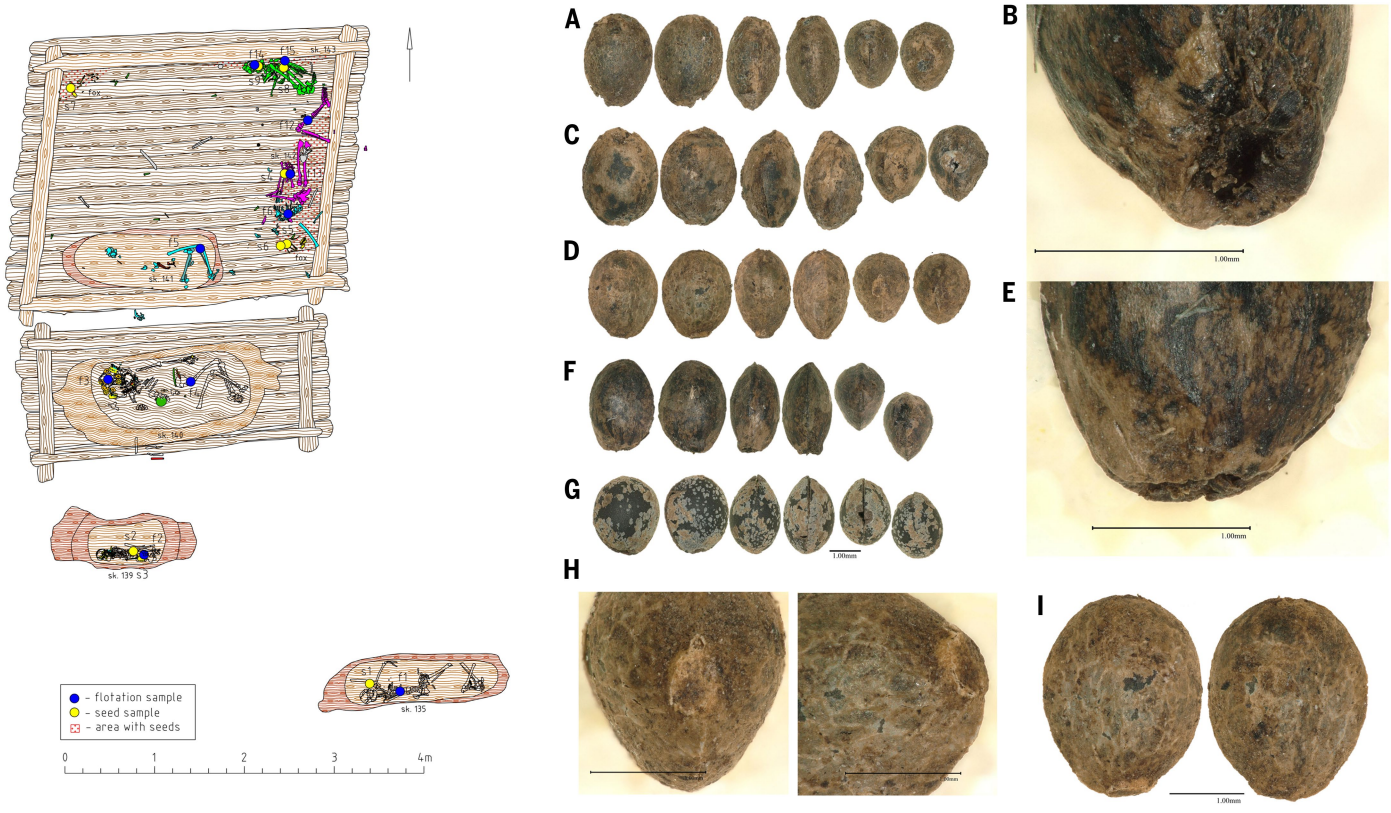


Fig. 2. The central burials at Tunnug 1 with sample locations. Cannabis seeds from the burials, with dendric vascular surface structure. Presented seeds were retrieved from various samples: (**A**) S01-01, [(**B**), (**E**), and (**F**)] S08-01, (**C**) S01-02, [(**D**), (**I**), and (**H**)] S02-01, and (**G**) S08-02. Scale bars, 1 mm; there is one scale for (A), (C), (D), (F), and (G).

Table 1. Direct radiocarbon dates from cannabis seeds, dated at the Curt-Engelhorn-Centre Archaeometry gGmbH.

Lab number	Uncalibrated	95.4% Probability	Numbers in Fig. 2
78291	2577 ± 19 BP	803–768 cal BC	S01
78292	2583 ± 19 BP	803–772 cal BC	S01
78293	2580 ± 19 BP	803–771 cal BC	S02
78294	2574 ± 19 BP	804–762 cal BC	S02
78295	2543 ± 19 BP	794–568 cal BC	S08
78296	2605 ± 20 BP	808–777 cal BC	S08

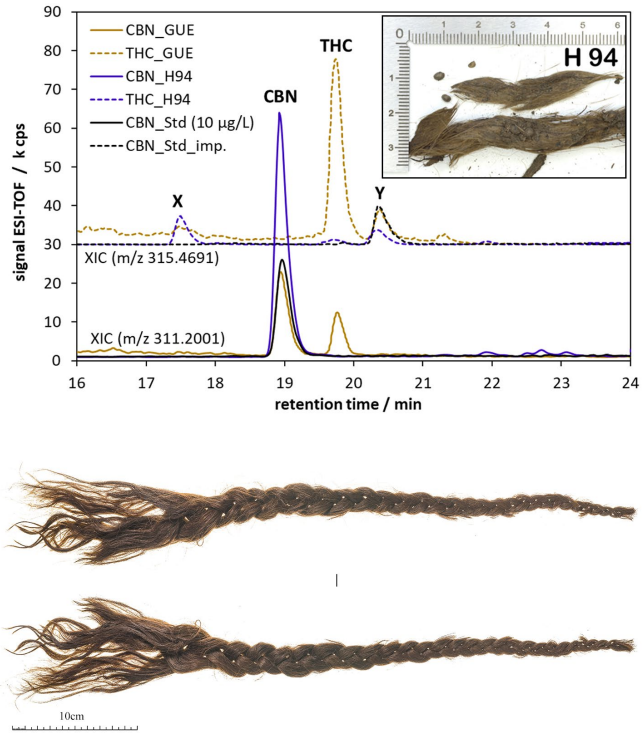


Fig. 3. Mass spectrometry results. (Top) Extracted ion chromatograms (XIC, ±10 ppm) of sample H94 extract (purple lines), a comparative sample of hair extract from a modern consumer (ochre lines), and a CBN reference standard (black lines) by LC-ESI-QTOF measurement. The picture in the blue frame shows sample H94 (scale in cm). Signals X and Y: unknowns matching the exact monoisotopic mass of THC (±10 ppm) but having deviating retention. (Bottom) Braided human hair sample recovered from the tomb.

could not be quantified. However, signals with identical retention time and matching tandem mass spectrometry (MS/MS) spectra were detected in hair samples of contemporary cannabis consumers. Further confirmation of both compounds was obtained by comparison with literature MS/MS data (supplementary materials). Quantitative determination of CBN was performed by reversed-phase chromatography coupled to a tandem mass spectrometer (LC-ESI-MS/MS). The CBN levels were up to 0.89 µg/g with the exceptions of enhanced contents in samples H4 (8.8 µg/g) and H94 (100 µg/g). The median of the CBN concentration in the prehistoric samples was 0.56 µg/g.

Discussion

Ritual, medicinal, and recreational uses of plants are notoriously difficult to identify in the archaeological record, because (i) the plant parts used tend to be highly perishable: leaves, flowers, and resins, as opposed to seeds, nuts, or fruit shells; (ii) they tend to be only occasionally used, whereas daily use items, such as grains, are far more likely to become incorporated into the archaeological record; and (iii) the nature of usage or consumption is difficult to prove on the basis of the presence of plant remains alone. Despite the limited evidence, it has long been argued that the earliest ritual use of cannabis comes from the Eurasian steppe, both because of the prominence of wild cannabis plants and owing to the historical account of Herodotus. Many scholars have debated over the antiquity of this early cannabis use, opening up questions such as when it was first cultivated and what role it played in prehistoric cultures (*8*, *18*, *33*–*36*). The application of mass spectrometry to archaeological hair is rare, but this is mostly because of a lack of available ancient hair. Testing hair for drug metabolites has a long history in clinical and forensic toxicology (*37*, *38*). Hair offers the advantage of a longer testing window for the drug, mostly limited by the length of the hair (with hair growing ~1 cm per month) (*39*). Although toxicological tests can confirm exposure, they are unsuitable for quantification of drug intake (*40*). Some studies also suggest that it is difficult to differentiate between active use and passive exposure to cannabis (*41*), although traces of metabolites in or on the tested hair still come from contact with other cannabis consumers or cannabis smoke.

The evidence that we were able to generate through hair analysis and the presence of plant parts in the tomb establishes a first case in which we have both the plant product and its biomolecular traces within the consumer tissue present in the same archaeological context. Although proving intent in a prehistoric context will remain impossible, the evidence is strongly in favor of intentional consumption for the plant's psychoactive properties. Even the unlikely unintentional ingestion or inhalation of psychoactive compounds constitutes psychoactive consumption. Already in the 1940s, the Soviet archaeologist Rudenko (*21*) excavated a Pazyryk burial mound in the Altai Mountains. The log chamber burials were encased in permafrost. Two copper alloy braziers containing stones were exhumed, accompanied by the parts for assembling a small tent and a leather flask full of cannabis seeds (*42*). Evidence for a similar mortuary tradition was recently identified by archaeological remains recovered from far western China in the Pamir Mountains at the Jirzankal burial complex. This site dates to 2500 years ago, and 10 of the burials in the cemetery contained wooden bowls that contained stones and showed evidence of burning (*8*). Although the plants did not preserve there, a metabolite analysis of the internal surface led to the identification of cannabinols. Both of these sites corroborate the narrative presented by Herodotus, whereby Scythian tribes were known to consume cannabis for psychoactive purposes (*22*).

Further supporting the importance of hemp among mortuary rituals in the montane regions of Inner Asia, a burial in the Jiayi Cemetery of Xinjiang contained 13 full cannabis plants draped over the body of an individual (*19*). A burial in the Yanghai Cemetery in the same region, dating to around 500 BCE, contained a woven leather basket and bowl filled with cannabis seeds, leaves, and stems (*20*, *43*). The cannabis remains from all three sites—Jirzankal, Yanghai, and Jiayi—have been argued to express higher psychoactive chemical compound levels than would be expected for wild species, although confirming this is not a straightforward task, as Ren *et al.* (*8*) point out. Written historical sources from China do not provide early references to cannabis use as a drug or ritual plant, but they do clarify that it has a deep history in East Asia as an oil-seed and fiber crop (*11*, *44*). Historians have linked words in Late Vedic Sanskrit to cultivated cannabis for both hemp and drug use. If the connection of cannabis with bhanga (intoxicating plant) in the Hymns of the Atharvaveda, mentioned along with soma, is correct, then it may suggest that the plant has been used as a drug in South Asia since about 1000 BCE (*17*). An additional recent discovery of cannabinol residues on a stone altar excavated in the 1960s from

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the site of Tel Arad in Israel further opens up the possibility for a wider geographic range over which the plant was used. The stone burners on the altar have been dated between 760 and 715 calibrated years (cal) BCE, and the residue has been interpreted to be mixed with frankincense (*45*). There is a long history of archaeologists and historians seeking to identify the Vedic plants, especially the mythical soma. The most cited defense of the identification of soma as cannabis came from Turkmenistan (*46*, *47*), but the identification has subsequently been disproven (*48*). Although the Tunnug 1 is located far away from the writers of the Vedic texts, the discovery gains importance in light of the long history of misidentifications and speculations about ancient drug use.

There are now several sites in the broader region where cannabis has been recovered from burials, and it is clear that ancient people were specifically using it in a funerary context. In several cases, the plants had been burned. Although we cannot rule out the possibility that this plant was also targeted as incense, its consumption through either ingestion or inhalation would have produced psychoactive effects independent of consumer intent. Furthermore, many far-more-aromatic plants would have been available for that purpose in the local ecosystem, such as *Artemisia* spp. and *Juniperus* spp. Additionally, the two uses are not mutually exclusive. The likely involvement of human sacrifice leading to the creation of a spectral cavalcade (*31*) would have potentially made the plant a useful agent to calm individuals who were to be included in the rituals and, thus again, contextually supports the intentional usage of the plant for its psychoactive properties. If the plants were used fresh, then the seeds would have been on the plants in late summer or early autumn. This would potentially add an additional benefit to using flowers in funerary rituals by surrounding a body with inflorescences, which could have partially covered the odor of decomposing flesh. Potentially, this could also be seen in the context of mummification practices, which are evidenced in later Pazyryk burials, where muscles and intestines were removed and the skin stuffed with plant matter [compare to (*49*–*51*)]. However, given the direct molecular evidence from human hair, such practices seem to be, at most, secondary to the plant's use as a drug.

The findings from Siberia represent the earliest securely dated direct evidence for psychoactive use of cannabis and considerably extend the known temporal scope of the practice back to 2800 years ago. Direct molecular evidence from human hair further corroborates that plant parts were consumed for their psychoactive properties within a ritual context. These data add to the growing global repertoire of archaeological evidence for ancient people using plants as mind-altering or mood-altering drugs. Early cannabis use thus emerges not simply as a pharmacological curiosity but as an indication of how ancient societies experimented with and ritualized the psychoactive qualities of the botanical world.

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SUPPLEMENTARY MATERIALS

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Editor's summary
Psychoactive plants are used globally today, with increasing evidence of medicinal value and, in some cases, decreasing illegality. Cannabis is one of the most widely used and cultivated of these plants. The prevalence of past usage, particularly for ritual purposes, has often been suggested, but making conclusions about its use for psychoactive properties has been difficult. Cannabis plants are ephemeral, and even the presence of plant parts and seeds could be an indication of cultivation for its many other useful properties. Caspari *et al*. found cannabis plant parts in a 2800-year-old tomb in Siberia and also identified its psychoactive compounds in hair found within the tomb, suggesting ritualistic psychoactive use. —Sacha Vignieri

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