Phytochemical and Genetic Analyses of Ancient Cannabis from Central Asia. *Ethan B. Russo, Hong-En Jiang, Xiao Li, Alan Sutton, Andrea Carboni, Francesca Del Bianco, Giuseppe Mandolino, David J. Potter, You-Xing Zhao, Subir Bera, Yong-Bing Zhang, En-Guo Lü, David K. Ferguson, Francis Hueber, Liang-Cheng Zhao, Chang-Jiang Liu, Yu-Fei Wang, & Cheng-Sen Li*

Supplementary Data

Fig S1. Study site at the Yanghai Tombs with Huoyan Shan mountain range in background (photo EBR).

A   B
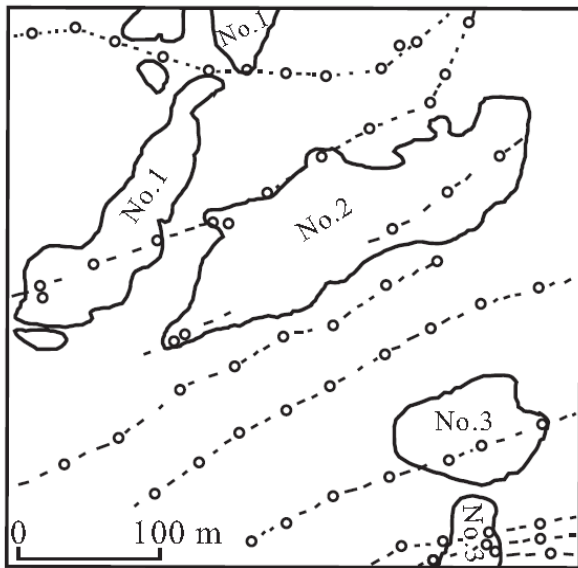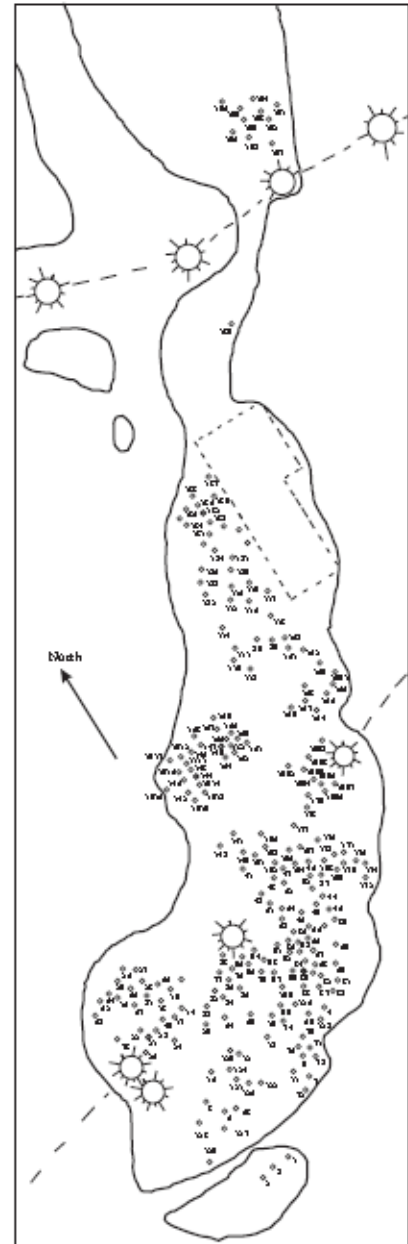

Fig S2. Diagrams of the Yanghai Tombs (adapted from (Xinjiang Institute of Cultural Relics and Archaeology, 2004), with permission). **A.** The subsections I-III span a time of 3200-2000 years BP. Circles along dotted lines represent *karez,* subterranean water tunnels employed by the Uighur peoples for irrigation, currently inactive. Section I measures 300 m in length by 50 m in width. **B.** Enlargement of Field I with Tomb M90 at lower portion.
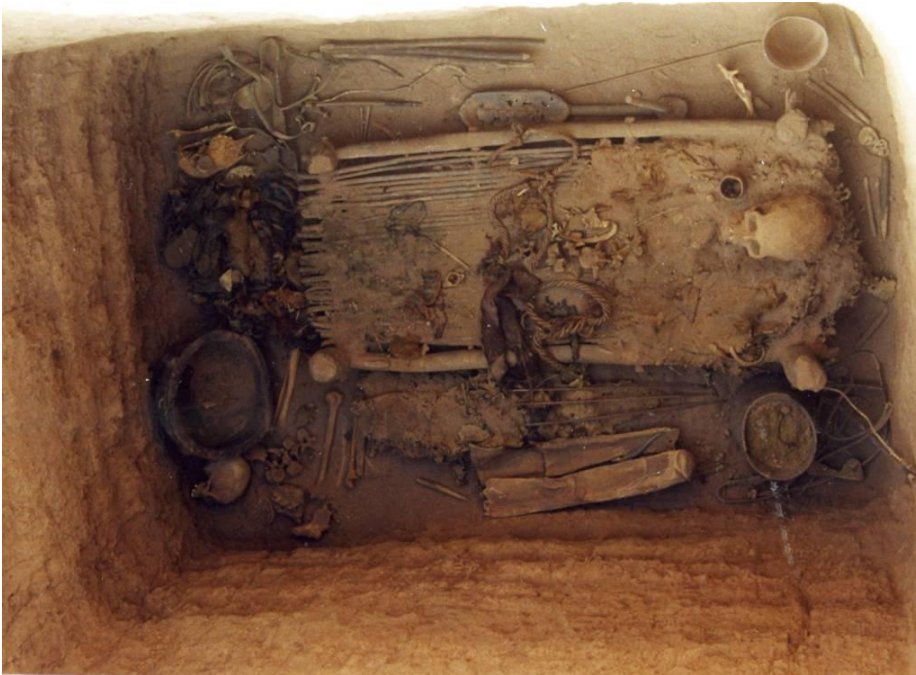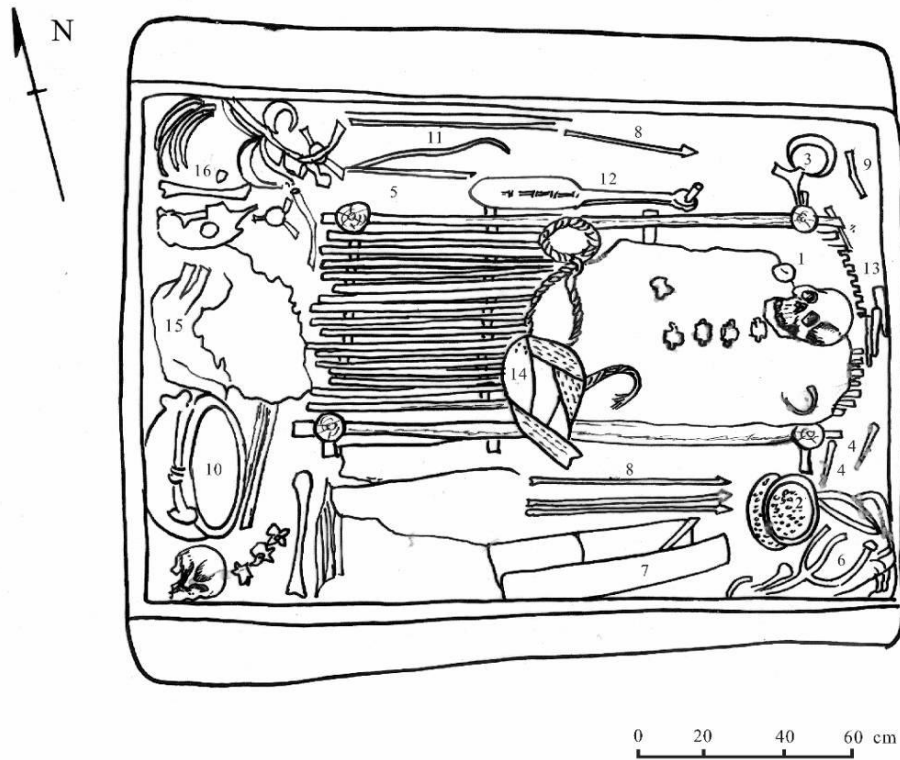
Fig S3A.

Fig S3B.

Fig S3. The shaman's tomb, M90 (previously published in Mandarin (Xinjiang Institute of Cultural Relics and Archaeology, 2004), used with permission). **A.** Photograph of the tomb, as excavated *in situ* in 2003. At top, a shelf is observed. This was covered with 10 cm. diameter tree branches and

grass with Gobi gravel deposited on top. Below, an air space was present down to the full 210 cm depth. The tomb length was 220 cm, and width 175 cm. **B.** Diagram of tomb and its contents. As noted in main text, the shaman was found as a disarticulated skeleton, with evidence of an apparent leg wound. The skull and bones at his feet were felt to be those of a female sibling. The tomb contents included: 1) earthenware pot, 2) leather basket containing cannabis, 3) earthenware pot, 4) wooden bar implements, 5) horse lash, 6) bridle or halter, 7) leather medicine bag, 8) bows and arrows, 9) wooden implement, 10) wooden bowl containing cannabis, 11) arrows 12) *kongou* harp, 13) wooden wimble tool to untie knots, 14) leather make-up bag, 15) woolen fabric, 16) leather ring hand protector for bowstring.

Fig S4A.

Fig S4B.

Fig S4C.

Fig S4. The shaman's skull (photos EBR). **A.** Anterior view. **B.** Lateral view. **C.** Close-up of teeth demonstrating a high degree of wear, attributable to possible high fiber diet and utilization of teeth in crafting techniques.

Fig S5A.

Fig S5B.

Fig S5. Containers in which cannabis was stored in tomb (previously published in Mandarin (Xinjiang Institute of Cultural Relics and Archaeology, 2004), used with permission).

A. Leather basket adjacent to skull (scale as per diagram). **B.** Wooden pot from foot of bier. Note lacunae worn through due to its employment as a mortar. Also visible are drilled holes to hold patching material to prevent spillage, but none was present in the tomb (scale as per diagram).
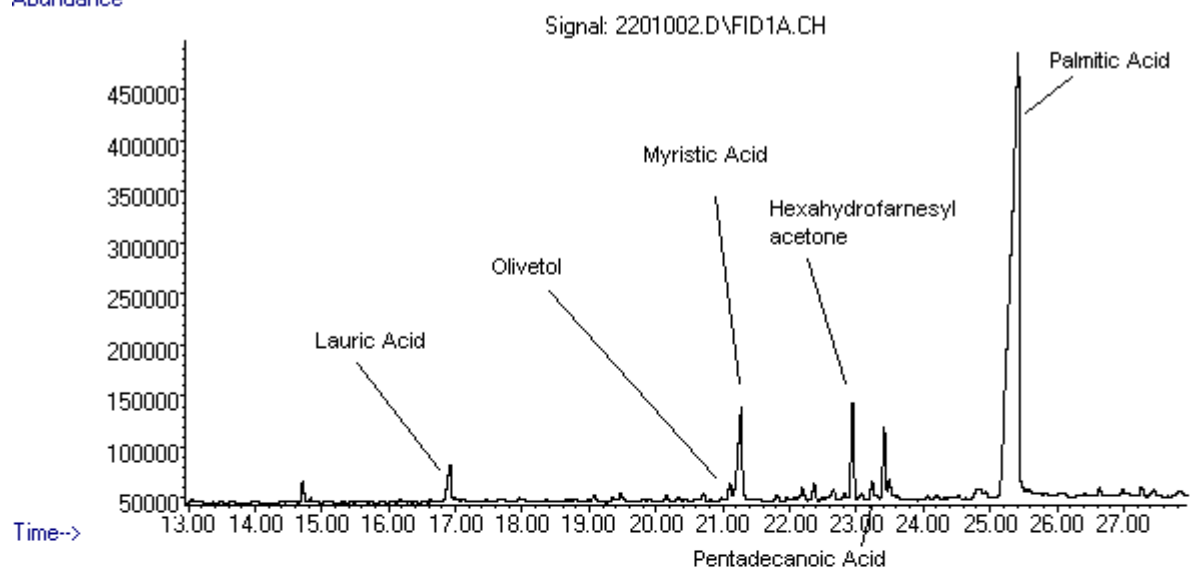
Fig S6A.

Fig S6B.

Fig S6C.

Fig S6. Re-excavation of Tomb M90. This was undertaken to re-examine artifacts, measure GPS coordinates, and assess environmental conditions (photos EBR). **A.** The tomb itself demonstrating upper shelf and inner chamber. The sides are formed of loess. The ground temperature in the SE corner of the tomb in the area where the leather basket of cannabis was located measured as 12°C by H.E.J. at the hottest part of the day at solar time 14:30 on 22 March 2008, just after the vernal equinox. The soil temperature outside the tomb at that time was 36.5°C, thus the gradient demonstrates the "old and cold" storage of the cannabis for 2700 years. The soil was highly alkaline. **B.** Woolen fabric removed from the tomb. Note the plain weave pattern. **C.** A piece of rope from the tomb, fabricated from *Phragmites* (reed) spp
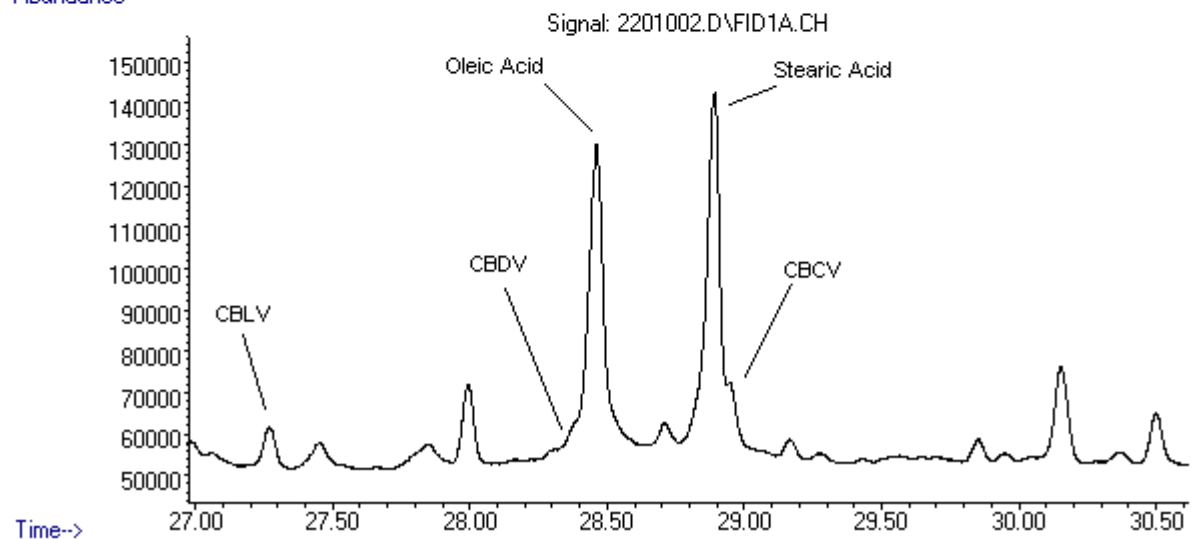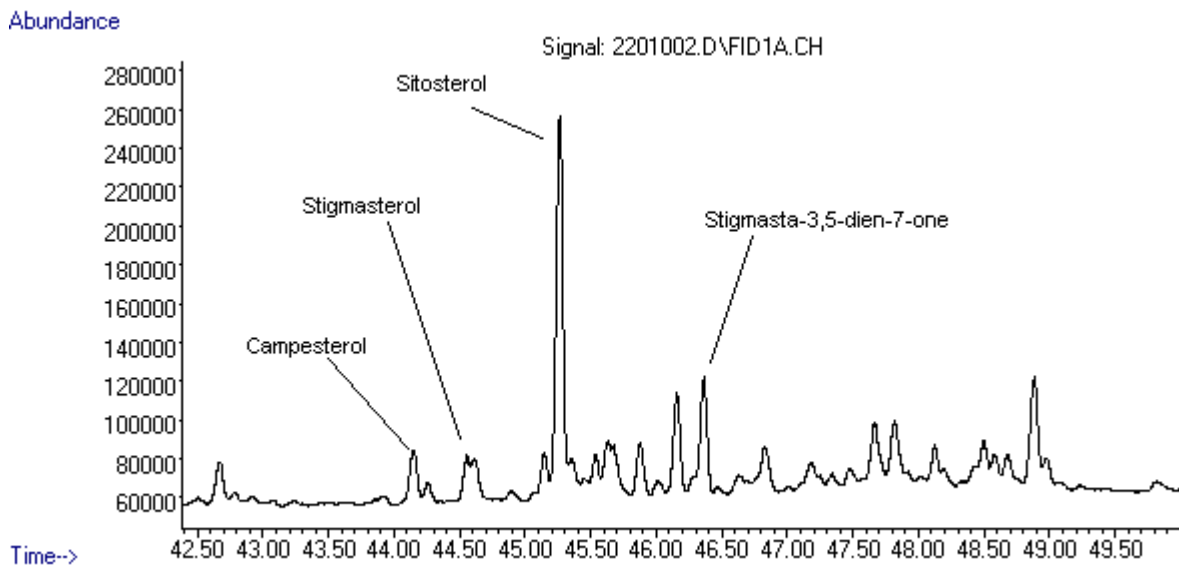
Fig S7A.

Fig S7B.

Fig S7. Chromatography Sub-sections from phytochemical analysis. **A.** 13-30.5 min region. Phthalate peaks are felt to be artifacts of storage of cannabis in polythene bag. Olivetol is precursor to terpenoid components of cannabis not otherwise seen herein (McPartland and Russo, 2001). **B.** 42-50 min region. This contains phytosterols and triterpene alcohols with beta-sitosterol as the most abundant component (McPartland and Russo, 2001).

Primer	Sequences
Fw150	ACTCGTATACACTCAACACGACCAA
Fw154	GTATACACTCAACACGACCAAT
Fw166	CACGACCAATTGTATATGTCTATC
Rev318	CTGCAAGCCAACTTTCTT
Rev328	GAGTTCGAATCTGCAAGCCA

Fig S8. Primer sequences employed in the genetic analysis to amplify THC- and CBD-allele specific fragments and their sequences (5' → 3').