UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

The ecological implications of a Yakutian mammoth's last meal

van Geel, B.; Aptroot, A.; Baittinger, C.; Birks, H.H.; Bull, I.D.; Cross, H.B.; Evershed, R.P.; Gravendeel, B.; Kompanje, E.J.O.; Kuperus, P.; Mol, D.; Nierop, K.G.J.; Pals, J.P.; Tikhonov, A.N.; van Reenen, G.; van Tienderen, P.

DOI
10.1016/j.yqres.2008.02.004

Publication date
2008

Document Version
Final published version

Published in
Quaternary Research

Link to publication

Citation for published version (APA):
van Geel, B., Aptroot, A., Baittinger, C., Birks, H. H., Bull, I. D., Cross, H. B., Evershed, R. P., Gravendeel, B., Kompanje, E. J. O., Kuperus, P., Mol, D., Nierop, K. G. J., Pals, J. P., Tikhonov, A. N., van Reenen, G., & van Tienderen, P. (2008). The ecological implications of a Yakutian mammoth's last meal. *Quaternary Research*, *69*(3), 361-376. https://doi.org/10.1016/j.yqres.2008.02.004

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, P.O. Box 19185, 1000 GD Amsterdam, The Netherlands. You will be contacted as soon as possible.
UvA-DARE is a service provided by the library of the University of Amsterdam (https://dare.uva.nl)

Download date:16 Oct 2026

Available online at www.sciencedirect.com
ScienceDirect
Quaternary Research 69 (2008) 361–376
QUATERNARY RESEARCH
www.elsevier.com/locate/yqres

The ecological implications of a Yakutian mammoth's last meal

Bas van Geel [a,*], André Aptroot [b], Claudia Baittinger [c], Hilary H. Birks [d], Ian D. Bull [e], Hugh B. Cross [f], Richard P. Evershed [e], Barbara Gravendeel [a,f], Erwin J.O. Kompanje [g], Peter Kuperus [a], Dick Mol [h], Klaas G.J. Nierop [a], Jan Peter Pals [i], Alexei N. Tikhonov [j], Guido van Reenen [a], Peter H. van Tienderen [a]

[a] *Institute for Biodiversity and Ecosystem Dynamics, Universiteit van Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands*
[b] *Adviesbureau voor Bryologie en Lichenologie, Gerrit van der Veenstraat 107, 3762 XK Soest, The Netherlands*
[c] *NEDL — North European Dendro Lab, Copenhagen, and Environmental Archeology, National Museum of Denmark, Copenhagen, Denmark*
[d] *Department of Biology, University of Bergen, N-5007 Bergen, and Bjerknes Centre for Climate Research, N-5007 Bergen, Norway*
[e] *Organic Geochemistry Unit, Bristol Biogeochemistry Research Centre, School of Chemistry, University of Bristol, Bristol, BS8 1TS, UK*
[f] *Nationaal Herbarium Nederland — Leiden University, Leiden, The Netherlands*
[g] *Erasmus MC University Medical Center Rotterdam, and Natural History Museum, Rotterdam, The Netherlands*
[h] *Natural History Museum, Rotterdam, The Netherlands*
[i] *Amsterdam Archaeological Centre, Universiteit van Amsterdam, Amsterdam, The Netherlands*
[j] *Zoological Institute, Russian Academy of Sciences, Saint Petersburg, Russia*

Received 3 May 2007
Available online 28 March 2008

Abstract

Part of a large male woolly mammoth (*Mammuthus primigenius*) was preserved in permafrost in northern Yakutia. It was radiocarbon dated to ca. 18,500 ^{14}C yr BP (ca. 22,500 cal yr BP). Dung from the lower intestine was subjected to a multiproxy array of microscopic, chemical, and molecular techniques to reconstruct the diet, the season of death, and the paleoenvironment. Pollen and plant macro-remains showed that grasses and sedges were the main food, with considerable amounts of dwarf willow twigs and a variety of herbs and mosses. Analyses of 110-bp fragments of the plastid rbcL gene amplified from DNA and of organic compounds supplemented the microscopic identifications. Fruit-bodies of dung-inhabiting Ascomycete fungi which develop after at least one week of exposure to air were found inside the intestine. Therefore the mammoth had eaten dung. It was probably mammoth dung as no bile acids were detected among the fecal biomarkers analysed. The plant assemblage and the presence of the first spring vessels of terminal tree-rings of dwarf willows indicated that the animal died in early spring. The mammoth lived in extensive cold treeless grassland vegetation interspersed with wetter, more productive meadows. The study demonstrated the paleoecological potential of several biochemical analytical techniques.
© 2008 University of Washington. All rights reserved.

Keywords: Ancient DNA; Biomarker; Diet; Dung; Lipid; *Mammuthus primigenius*; Microfossils; Macro-remains; Tree-ring analysis; Yukagir

Introduction

Woolly mammoths, *Mammuthus primigenius* (Blumenbach, 1799), roamed unglaciated Siberia during the last glacial period and are occasionally preserved in permafrost. In 2002 the head, front legs and parts of the stomach and intestinal tract of the Yukagir Mammoth (YM) were discovered in the permafrost on the steep side of an oxbow lake near the Maxunuokha River in northern Yakutia (Sakha Republic), Russia (GPS 71° 52' 988" North – 140° 34' 873" East; Fig. 1). Five radiocarbon dates of the YM are available (Table 1). The average of the measurements calibrates into ca. 22,500 cal yr BP (Mol et al., 2005, 2006a). The remains of the YM are stored in the collection of the Mammoth Museum in Yakutsk, the capital of the Sakha Republic (Yakutia). The museum forms part of the Institute of

* Corrresponding author. Fax: +31 20 5257832.
E-mail address: vanGeel@science.uva.nl (B. van Geel).

0033-5894/$ - see front matter © 2008 University of Washington. All rights reserved.
doi:10.1016/j.yqres.2008.02.004

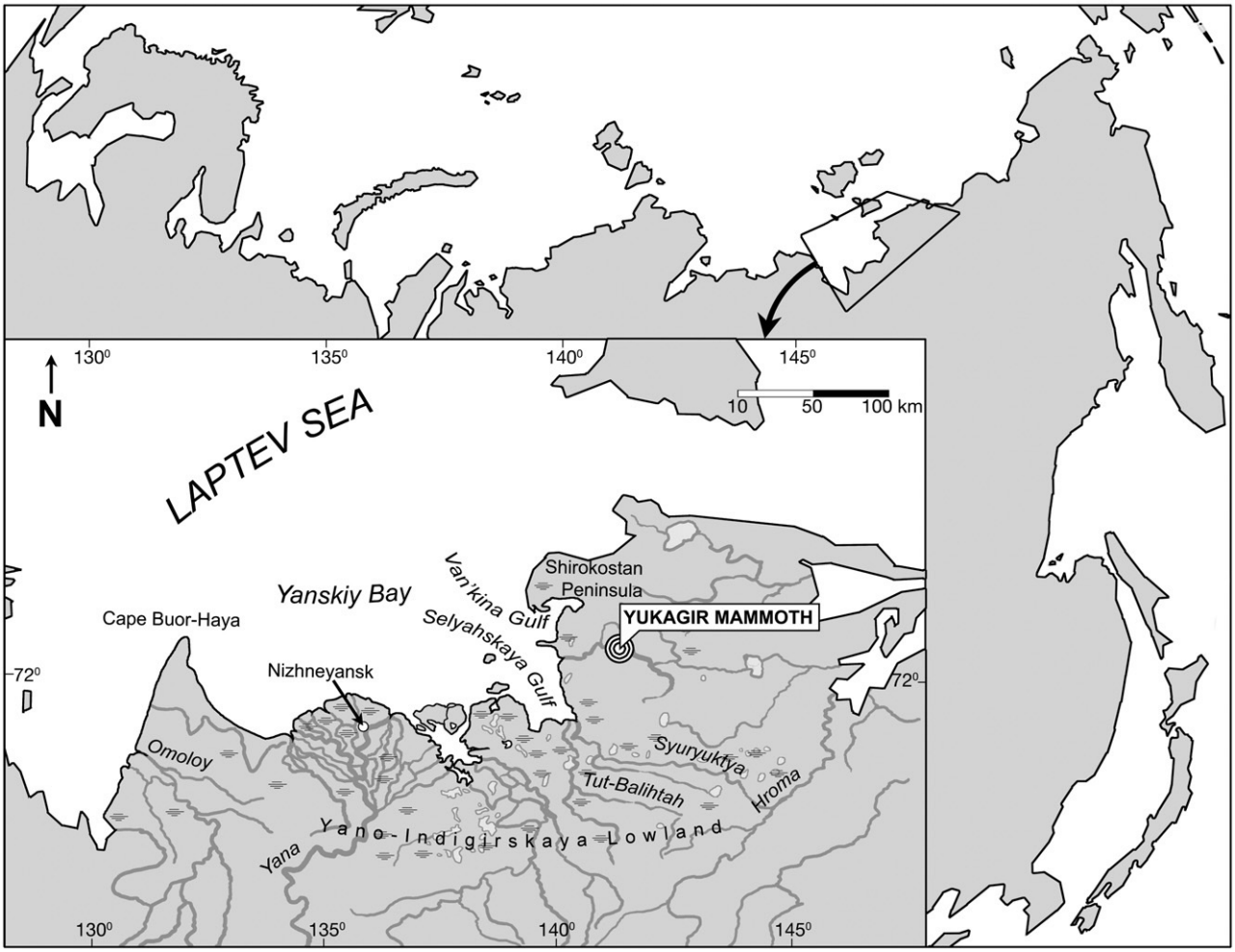

Figure 1. Location map.

Applied Ecology of the North, Academy of Sciences of the Sakha Republic.

The head of the YM is extremely well preserved (Fig. 2, 1) compared to the other parts of the carcass; almost the entire head, including the mandible, is still covered with thick skin and the mouth is closed. Consequently, the molars in the upper and lower jaws are not exposed, and it is not possible at this stage to inspect the molars without inflicting damage. Computed tomography scans, made in Japan, indicate that the last molars, m3/M3 left and right are still in place but they are heavily worn. The pelvic girdle could have provided confirmation of the gender but it was not preserved. Based on the size and the curvature of the tusks, however, the YM could be identified as a male individual (D.C. Fisher, Michigan, USA, personal communication).

The YM was average-sized for a male woolly mammoth. It had large spirally twisted tusks, typical of an old individual. Based on the measurements of the entire left front leg in anatomical position, the shoulder height is estimated to have been 272 cm, including 13 cm added for missing soft tissue at the shoulder. However, based on combined data from the entire left front leg and the forefoot circumference, the estimated shoulder height is 283 cm. From the estimated height and data from the humerus the estimated weight of the YM is between 4 and 5 tons (Mol et al., 2007; Shoshani and Mol, 2007).

The YM's last meal was preserved in its intestinal tract. The habitats of ice-age mammoths and other herbivores are broadly known from vegetation reconstructions from preserved gut contents and dung with complementary information coming from plant macrofossils and pollen from sediments (Solonevich et al., 1977; Ukraintseva, 1979; Gorlova, 1982a,b; Ager, 2003; Liu and Li, 1984; Davis et al., 1985; Mead et al., 1986; Ukraintseva, 1993; Vasil'chuk et al., 1997; Kienast et al., 2001, 2005; Zazula et al., 2003, 2006a,b; Sher et al., 2005; Mol et al., 2006b; Kienast, 2007;

Table 1
Radiocarbon dates of the Yukagir Mammoth (after Mol et al., 2005)

Field no	Sample	Laboratory no	Results (^{14}C yr BP)
CM-DM-64	bone (rib)	GrN-28258	18,510±80
CM-DM-64	idem	AA-59602	18,160±110
CM-DM-65	skin	GrN-28259	18,510±100
CM-DM-66	hair	GrA-24288	18,680±100
–	bone	GIN-12719	18,200±60

GrN: conventional radiocarbon dating Groningen, Netherlands; GrA: AMS radiocarbon dating Groningen; AA: AMS radiocarbon dating, Tucson, Arizona, USA; GIN: conventional radiocarbon dating Moscow, Russia. The average of the measurements calibrates into ca. 22,500 cal yr BP.

Figure 2. 1: Head of the Yukagir Mammoth (YM); 2: Dung from the intestine of the YM showing *Salix* twigs.; 3–11: pollen. 3: Poaceae; 4: *Artemisia*; 5: Caryophyllaceae; 6: *Polemonium*; 7: *Polygonum persicaria* type; 8: *Armeria* type; 9: Asteraceae tubuliflorae; 10: Asteraceae liguliflorae; 11: *Epipactis*; 12: *Salix* twigs; 13: thin *Salix* twigs with axillary buds; 14: *Salix* fruits; 15: *Salix* leaf remains.

Drescher-Schneider et al., 2007). Additional information about climate, environment and diets is based on stable isotope studies of mammoth tusks, teeth and hairs and bones of Pleistocene herbivores and carnivores (Iacumin et al., 2006; Rountrey et al., 2007; Tütken et al., 2007; Fox-Dobbs et al., in press). It has been proposed that Beringia, the vast east-Siberian west-Alaskan area linked by the Bering Land Bridge, was vegetated by an extinct biome, the so-called 'mammoth steppe' (Guthrie, 1990, 2001) with the centre of the land bridge probably being moister than the more arid continental regions on either side. However, more detailed studies using a variety of proxies indicate that the vegetation was not uniform but locally differentiated into communities under the influence of factors such as exposure or shelter influencing snow-lie and water availability (Elias et al., 1996; Kienast et al., 2001, 2005; Yurtsev, 2001; Goetcheus and Birks, 2001; Zazula et al., 2006b).

Several recent studies show that DNA extraction and amplification from paleofeces is a promising new tool for dietary analysis of extinct mammals (Hofreiter et al., 2000, 2003; Kuch et al., 2002). Sequences of plastid DNA can supplement information from pollen and macrofossils and have the potential to provide more detailed information in cases where plant remains cannot be identified using morphological characteristics due to drastic modification by masticatory and digestive processes, or due to an unspecific seed or pollen morphology. Amplification of relatively short DNA fragments can result in detection of plants missed by conventional detection methods (Rollo et al., 2002; Willerslev et al., 2003). DNA analysis of fossil dung can thus provide a more diverse picture of dietary habits of extinct animals and also improve our understanding of their ecology (Hofreiter et al., 2003). Investigation of the lipid and macromolecular components extant within the organic fraction of the dung material can also enable inferences to be drawn about the botanical composition (since these components will be strongly influenced by the composition of epicuticular waxes and structural biopolymers derived from the dietary vegetation) as well as the effects of digestion and decomposition processes and the relative level of preservation of the remaining organic matter.

We present information about the physical condition of the YM and we focus our study on the dung from its lower intestinal tract. We used an array of microscopic, chemical and molecular techniques to reconstruct the diet, season of death, and the paleoenvironment. Our results are compared with other Russian mammoth dung samples from different times during the glacial period.

Materials and methods

Osteology and botanical microscopy

The excavated bones were investigated in order to determine the physical condition of the YM. Microfossil and macrofossil samples, and also the samples for organic chemistry and ancient DNA were obtained with clean tools from the inner part of the compact lump of dung that was taken from the intestinal tract. Sub-samples for the analysis of pollen and other microfossils were prepared according to Fægri and Iversen (1989). Pollen and other microfossils were identified and counted, including fungal spores from coprophilous Ascomycetes (Aptroot and van Geel, 2006; van Geel and Aptroot, 2006). For macrofossil analysis ca. 240 ml of the dung material was gently boiled in 5% KOH and sieved (meshes 120 μm). The material was suspended in water in a petri dish and examined systematically under a Leica MZ stereomicroscope (Birks, 2001). Identifications were made with the help of literature and reference collections of pollen, seeds, and vegetative plant remains. The annual rings of the willow twigs were studied by micro cross-sections stained with 1% safranin, mounted on slides, and preserved with Aquamount (Gurr®).

Chemistry of the dung material

Sample pre-treatment

Sub-samples were air-dried at 30°C. Willow branches were separated from the rest of the dung. Part of the samples was crushed with a mortar and pestle to measure organic carbon (OC) and total nitrogen (N_{tot}) using an Elementar VarioEL.

Extraction of lipids

Whole mammoth dung samples (100–200 mg) were Soxhlet extracted using dichloromethane/methanol (DCM/MeOH) (9:1 v/v) for 24 h. The residues were air-dried and used for further analysis. The total extracts were purified and analysed by gas chromatography/mass spectrometry (GC/MS) as described by Nierop et al. (2006). Fecal biomarkers were isolated for analysis from additional dung following the methods of Bull et al. (1999b) and a modified version of the methodology proposed by Elhmmali et al. (1997) for sterol and bile acid fecal biomarkers.

Gas chromatography/mass spectrometry (GC/MS)

On-column GC/MS analyses were performed using a ThermoQuest Trace GC 2000 gas chromatograph equipped with a 30 m Rtx-5Sil MS column (Restek) with an internal diameter of 0.25 mm and film thickness of 0.1 μm. Derivatised extracts (1.0 μl) in cyclohexane were injected on-column. The oven temperature was programmed from 100°C (isothermal for 2 min) to 130°C at 20°C/min and from 130°C to 320°C (isothermal for 20 min) at 4°C/min. The column was coupled to a Finnigan Trace MS quadrupole mass spectrometer operating at 70 eV and 250°C, scanning the range *m/z* 50–650 with a cycling time of 0.65 s. Quantification of alkanes, alkanols and alkanoic acids was performed by using the corresponding deuterated standards assuming a response factor of 1.0. Fecal biomarkers were analysed using an identical instrument equipped with a 60 m ZB1 column (Phenomenex) with an internal diameter of 0.32 mm and film thickness of 0.1 μm. Derivatised extracts dissolved in hexane were introduced (1.0 μl) via a programmable temperature vaporising injector. The oven temperature was programmed from 40°C (isothermal for 2 min) to 200°C at 10°C/min and from 200°C to 300°C (isothermal for 20 min) at 3°C/min for the sterol analyses and from 40°C (isothermal for 2 min) to 230°C at 20°C/min and from 230°C to 300°C (isothermal for 20 min) at 2°C/min for the

bile acid analyses. All compounds were quantified by GC-FID (HP 5890 Series II) using the same column and temperature programmes listed above with reference to an appropriate internal standard compound.

Pyrolysis-gas chromatography/mass spectrometry

Analytical pyrolysis consists of the thermal degradation of macromolecules in an inert atmosphere yielding low molecular weight fragments that can be subsequently analysed by GC/MS (Wampler, 1999). The product of pyrolysis, the pyrolysate, is considered to reflect the overall composition of the macromolecular fraction of the sample analysed. Pyrolysis was carried out using the method of Nierop and Verstraten (2004).

Thermally assisted Hydrolysis and Methylation (THM)

One drawback of conventional pyrolysis is that some polar compounds are not amenable to GC. To overcome this problem, *in situ* derivatization of the products during pyrolysis is applied to analyse these polar compounds. With Thermally assisted Hydrolysis and Methylation (THM), hydrolysable bonds are cleaved and the resulting carboxylic acid and hydroxyl groups are *in situ* transformed into their corresponding methyl esters and methyl ethers (e.g. Challinor, 2001). Apart from the methylation, the degradation mechanisms of macromolecules differ from conventional pyrolysis as well, and both techniques are therefore complementary. THM was carried out following the method of Nierop and Verstraten (2004).

DNA extraction, PCR amplification and sequencing

Dung samples were air-dried and stored at 4°C. Aliquots of 100–200 mg were dissected with a forceps and subsequently ground to fine powder in liquid nitrogen in a grinder mill (Retch). CTAB buffer (2% CTAB, 2% PVP, 20 mM EDTA, 100 mM Tris–HCl, pH 8.0, 1.42 M NaCl, 2% 2-mercaptoethanol) was added to 100 mg of powdered sample and incubated for 2 h at 65°C under agitation. DNA was extracted twice using an equal volume of chloroform:isoamyl alcohol (24:1), precipitated with 96% ice-cold ethanol and resuspended in TE buffer (contains Tris, a common pH buffer, and EDTA, a molecule chelating cations like Mg^{2+}; the purpose of TE buffer is to protect DNA or RNA from degradation). The suspension was reprecipitated with 96% ethanol in the freezer for 1 h, washed twice in 76% ethanol, and the pellet was then air-dried and resuspended in TE. Subsequently, an aliquot of each extraction was further purified using the Qiagen PCR purification kit, following manufacturers instructions. The final DNA extract was eluted from the silica columns using TE buffer after a 30 min incubation. All extractions were carried out in the special ancient DNA facility of Leiden University following established protocols to avoid contamination (Cooper and Poinar, 2001) and they were partly replicated in laboratories at Amsterdam University.

Amplifications were performed in an MJ Research thermal cycler (Biozym, Oldendorf, Germany) with a 3 min activation step at 94°C, followed by 35–60 cycles at 95°C for 30 s, 55°C for 60 s, and 72°C for 45 s. Primers rbcLA1a and rbcL19b (Hofreiter et al., 2000) were used in 25 microliter reactions. All amplification products were cloned using pGEM-T Easy Vectors (Promega) and 3–15 clones were sequenced from each amplification. All polymerase chain reactions were carried out in laboratories in Leiden physically separated from the ancient DNA facility with some replications at laboratories of Amsterdam University and always including extraction blanks to monitor contamination. DNA sequences obtained were compared with data in the NCBI GenBank using BlastSearch. Identifications were only accepted in cases where the GenBank sequences covered all 110-bp of PCR products obtained and showed no more than a single nucleotide difference.

Results

Physical condition of the Yukagir Mammoth

The YM had backbone problems. Thoracic vertebrae IV and V showed abnormal growth, possibly as a result of an autoimmune reaction to an inflammation somewhere else in the body. Only the thorn-shaped extremities of the two subsequent thoracic vertebrae have been retrieved; these were naturally cut off just above the neural canal and were strongly deformed, showing some pus channels. The available vertebrae before and after these pathologically modified specimens were in good condition. The YM suffered a form of spondylarthropathy (also known as ankylosing spondylitis, or rheumatoid spondylitis) in the 4th and 5th thoracic vertebrae. Unfortunately the pelvis bone and the sacrum bone are missing. Generally this disease shows most clearly in the joint between these two bones. Spondylarthropathy includes a group of inflammatory diseases comprising Reiter's syndrome, reactive arthritis, psoriatic arthritis and arthritis associated with inflammatory bowel disease. The bony outgrowths found on the vertebrae of affected individuals are called syndesmophytes (François et al., 1995). These are slim, bony outgrowths, parallel to the vertebral column, which replace the outer parts of the annulus fibrosus (part of the intervertebral disc) and the shorter and longer perivertebral ligaments, thus leading to an intervertebral bridge by means of complex processes involving ossification. The syndesmophytes can be distinguished from the vertical and chunky osteophytes (bone spurs) in degenerative vertebral disease, and the often bizarre new bone formation is associated with primary bacterial infections. The abnormal bony outgrowths on two thoracic vertebrae of the YM resemble the syndesmophytes usually found in spondylarthropathy in man and other mammals (Rothschild and Rothschild, 1994; Kompanje, 1999; Kompanje et al., 2000). A diagnosis of reactive spondylarthropathy, most probably associated with inflammatory bowel disease seems plausible in this case. The inflammations would have caused pain, especially in the early stages of abnormal bone growth, but this was most likely not related to death. The event or condition triggering this growth might have occurred several years earlier. Further study of structural and compositional variations in the growth pattern of the tusk of the YM by D.C. Fisher may show further evidence for this. Mol et al. (2003) reported a similar case of abnormal bony outgrowths on vertebrae of the Markel mammoth.

Botanical microfossils, macro-remains and ancient DNA

Pollen in the dung probably integrates several years of vegetation, being ingested from pollen deposited on plants, soil, and in water, as well as from the primary source, flowers. The pollen assemblage (Table 2; Fig. 2, 3-11) shows a typically biased taxa distribution, with a dominance of abundantly produced wind-dispersed pollen derived from Poaceae and *Artemisia* and much smaller amounts of insect-transported pollen from herbs such as *Armeria, Polemonium*, Caryophyllaceae, and Asteraceae. The absence of tree pollen shows that the vegetation was treeless throughout a wide region. Similar assemblages were recorded from the Taimyr peninsula (Andreev et al., 2003; Mol et al., 2006b), and from Bykovsky Peninsula near the Laptev Sea Coast (Sher et al., 2005). The inference of a 'mammoth steppe' biome was made from just such pollen assemblages (Guthrie, 1990).

Table 2
Percentages of pollen and spores in the colon contents of the Yukagir Mammoth

Angiosperms			
Order	Family	Genus/species	Percentage
Apiales	Apiaceae	Indet	0.3
Asparagales	Orchidaceae	*Epipactis*	+
Asterales	Asteraceae	*Artemisia*	16.0
		Liguliflorae	0.2
		Tubuliflorae	0.2
Brassicales	Brassicaceae	Indet	0.7
Caryophyllales	Amaranthaceae (Chenop.)	Indet	0.1
	Caryophyllaceae	Indet	4.7
	Plumbaginaceae	*Armeria* type	+
	Polygonaceae	*Polygonum persicaria* type	0.3
		Rumex acetosella type	0.8
Ericales	Indet		0.1
	Primulaceae	*cf. Androsace*	0.2
	Polemoniaceae	*Polemonium*	0.2
Fabales	Fabaceae	Indet	1.4
		Lotus	0.2
Lamiales	Plantaginaceae	*Plantago*	0.8
Liliales	Liliaceae	Indet	+
Malpighiales	Salicaceae	*Salix*	0.2
Poales	Cyperaceae	Indet	0.1
	Poaceae	Indet	70.6
Ranunculales	Papaveraceae	*Papaver*	0.1
	Ranunculaceae	Indet	0.7
Rosales	Rosaceae	*Potentilla* type	0.9
		cf. Rubus chamaemorus	1.9
		Sanguisorba officinalis	0.1
		Indet	0.2
pollensum			1914

Fungi			
Order	Family	Genus	Percentage
Sordariales	Lasiosphaeriaceae	*Cercophora* type	+
		Podospora type	+
	Sordariaceae	*Sordaria* type	0.8
Pleosporales	Sporormiaceae	*Sporormiella* type	4.7

Algae			
Order	Family	Genus	Percentage
Zygnematales	Zygnemataceae	*Spirogyra*	+
Sphaeropleales	Hydrodictyaceae	*Pediastrum*	0.1

Table 3
Macrofossils in the colon contents of the Yukagir Mammoth

Angiosperms			
Order	Family	Genus/species	Remains
Asterales	Asteraceae	*Achillea/Petasites*?	fruits
Brassicales	Brassicaceae	*Draba sp.*	seeds
Caryophyllales	Caryophyllaceae	*Sagina intermedia* type	seeds
	Amaranthaceae (Chenop.)	Indet	seeds
Ericales	Primulaceae	*Lysimachia sp.*	seed
	Polygonaceae	*Rumex acetosella*	fruit
		Rumex sp.	fruit
Malpighiales	Salicaceae	*Salix* cf. *arctica*	twigs, axillary buds, leaf remains, fruits
		Salix sp.	
Poales	Cyperaceae	*Carex dioica* type	fruits
		C. sp. trigonous	fruits
		C. nardina type	fruits
		cf. *Kobresia*	fruits
	Juncaceae	*Juncus sp.*	seeds
	Poaceae	cf. *Agrostis sp.*	fruits,
		Glyceria sp.	fruits
		Hordeum sp.	fruits
		Poa cf. *arctica*	fruits
		Indet	stems
Ranunculales	Papaveraceae	*Papaver* sect. *Scapiflora*	seeds
	Ranunculaceae	*Caltha palustris*	seeds
		Ranunculus cf. *nivalis*	fruits
		Ranunculus cf. *pygmaeus*	fruits
Rosales	Rosaceae	*Potentilla sp.*	seeds
		P. hyparctica type	seeds

Bryophytes			
Order	Family	Genus/species	Remains
Bryales	Bryaceae	*Bryum sp.*	plant
Hypnales	Amblystegiaceae	*Drepanocladus aduncus*	plant
Hypnales	Entodontaceae	*Entodon concinnus*	plant
Polytrichales	Polytrichaceae	*Polytrichum alpinum*	plant
Pottiales	Pottiaceae	Indet	plant

Fungi (ascomata with spores)			
Order	Family	Genus/species	Remains
Pleosporales	Lophiostomataceae	*Lophiostoma corticolum*	spores
	Pleosporaceae	*Pleospora herbarum*	spores
	Sporormiaceae	*Sporormiella sp.*	spores
Sordariomycetes	Magnaporthaceae	*Pseudohalonectria lignicola*	spores

The botanical macrofossils consisted of mostly well-preserved plant material, with fruits and seeds and moss remains of a variety of taxa (Table 3 and Figs. 2–4). The spectrum and abundance of taxa are strongly influenced by the food preferences of the mammoth during his last meal. Twigs of willows (*Salix* sp.) formed an important component of the meal (Fig. 2, 12). A certain identification of the species of the willow remains was not possible, but it was evident that small-leaved dwarf species had been consumed. The willow twigs were generally 1–2 (<4) mm

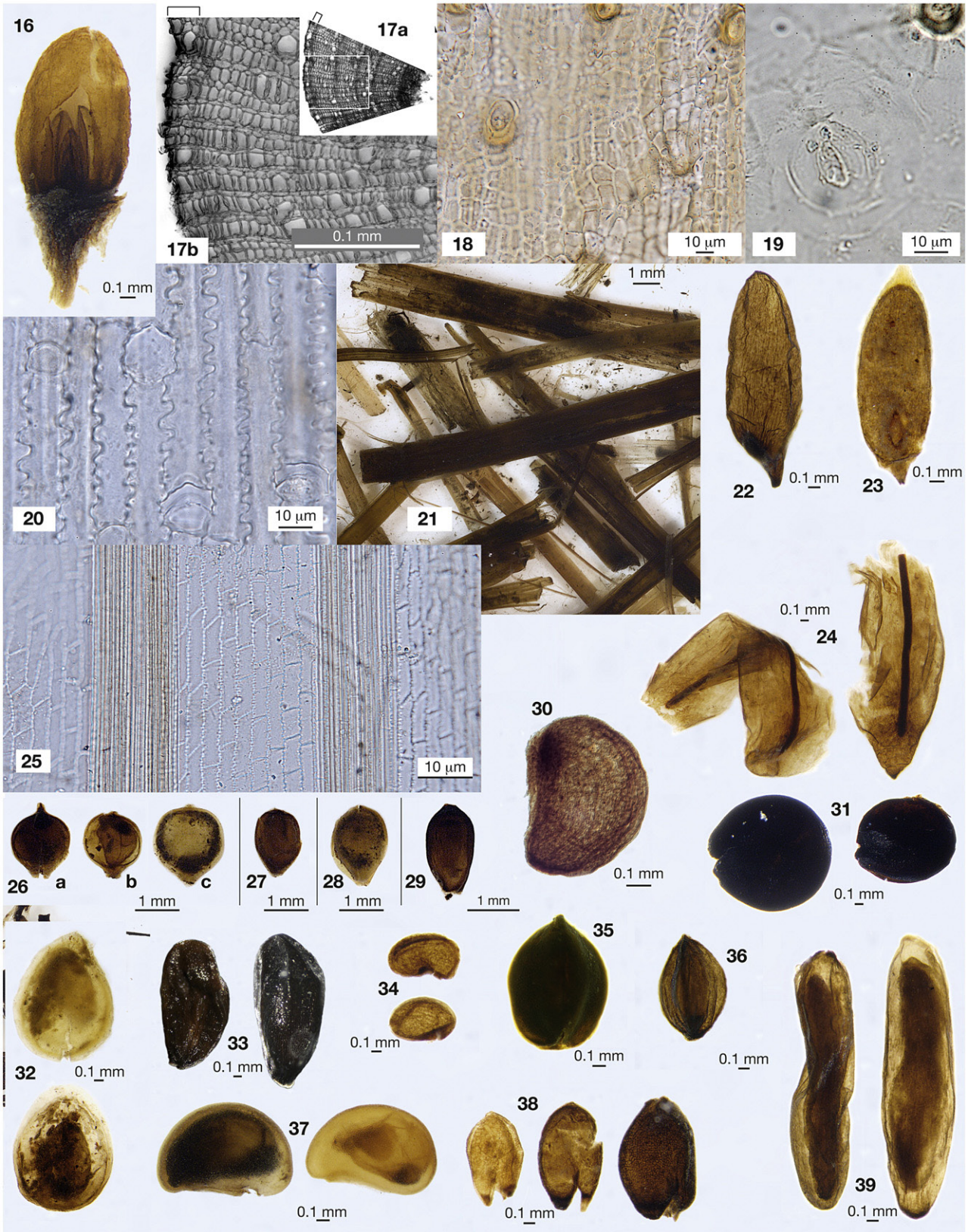

Figure 3. 16: Axillary bud of *Salix* with primordium inside; 17: Cross-section of a twig of *Salix* cf. *arctica*. The terminal tree-ring is incomplete, showing early-wood vessels formed in the beginning of the growing season, while the latewood is missing, indicating that the twig was browsed in early spring; 18: *Salix* epidermis; 19: *Salix* epidermis with stoma; 20: epidermis of Poaceae; 21: stems of Poaceae; 22: seed of *Poa* cf. *arctica*; 23: seed of *Agrostis* sp.; 24: seeds of *Hordeum* sp.; 25: epidermis of Cyperaceae; 26a–c: *Carex bicolor* type; 27: *Carex nardina* type; 28: *Carex dioica* type; 29: cf. *Kobresia*; 30: *Papaver* sect. *Scapiflora*; 31: Chenopodiaceae spp; 32: *Ranunculus* cf. *nivalis*; 33: *Caltha palustris*; 34: *Sagina intermedia* type; 35: *Rumex* sp.; 36: *Rumex* cf. *acetosella*; 37: *Potentilla*; 38: Brassicaceae (*Draba*); 39: cf. Asteraceae (*Achillea* or *Petasites*?).

in diameter, and up to 7.5 cm in length. Thicker twigs had more than 20 annual rings. The tree-ring structure and the diffusion, number, and size of the early wood vessels were compared to twigs from modern dwarf willow species and the morphological characteristics point to *Salix* cf. *arctica*. The growth rate of the willows is low compared to modern material of *S. arctica* from Northeast Greenland, indicating a harsher environment for the vegetation where the YM browsed. We studied nine twigs from the intestinal tract of the YM. The annual growth rate of twigs with 5 to ca. 25 rings (average number of annual rings: 13.1) is ca. 0.09 mm/year (standard deviation: 0.0264). The annual growth rate of recent material of *S. arctica* from Northeast Greenland is ca. 0.13 mm/year (standard deviation: 0.049; Schmidt et al., 2006), if we consider comparable material — twigs and small stems with 8–23 annual rings (average number of annual rings: 18.39, based on 23 individuals).

Thinner twigs showed scars where leaf stalks had been attached and many axillary buds were still connected to the twigs (Fig. 2, 13). In addition, fruits, many detached axillary buds, and leaves of *Salix* were recorded (Fig. 2, 14 and 15). Some buds contained well developed leaf primordia (Fig. 3, 16). The preservation of leaves was poor; in most cases only fragments of the venation were detected. Rarely were leaf cuticles preserved and these showed the characteristic *Salix* cell pattern (Fig. 3, 18–19). Stem fragments of grasses (Poaceae; Fig. 3, 20 and 21) and other monocotyledons (Fig. 3, 25) were the other major component of the dung.

Fruits and seeds of numerous monocotyledons were identified (Fig. 3, 22–24, 26–29), including *Poa* cf. *arctica*, cf. *Agrostis*

Figure 4. 40: unidentified stem fragments; 41: moss encrusted with soil dust; 42: calyptra; 43: moss sporangia; 44: *Polytrichum alpinum*; 45: *Entodon concinnus*; 46: *Drepanocladus aduncus*; 47: Pottiaceae.

Table 4
Plant identifications from the Yukagir mammoth dung based on 110-bp fragments of the plastid rbcL gene

Number of clones	Order	Family	Genus/species
5	Asterales	Asteraceae	*Achillea* *Bellis* *Leucanthemum*
4	Ericales	Primulaceae	Indet
3	Fabales	Fabaceae	*Astragalus*
3	Malpighiales	Euphorbiaceae	Indet
15		Salicaceae	*Salix*
3	Poales	Poaceae	*Deschampsia*
4	Ranunculales	Ranunculaceae	*Caltha palustris*
3	Rosales	Rosaceae	Indet

sp., cf. *Hordeum* sp., *Glyceria* sp., *Carex* spp., cf. *Kobresia*, and *Juncus.* Dicotyledon herbs (Fig. 3, 30–39) included *Potentilla* spp. including *P. hyparctica* type, *Rumex acetosella*, *Papaver* sect. *Scapiflora*, Caryophyllaceae including *Sagina/Minuartia*, Brassicaceae including *Draba*, Primulaceae (*Lysimachia*?), at least three species of Chenopodiaceae, a small *Ranunculus pygmaeus* type and *R.* cf. *nivalis, Caltha palustris*, and probably Asteraceae. The remains of mosses (*Drepanocladus aduncus*, *Polytrichum alpinum*, *Entodon concinnus*, *Bryum* sp. and Pottiaceae; Fig. 4, 41–47) indicate environments ranging from moist to dry conditions.

The mammoth dung yielded ten different plant DNA sequences (Table 4). Identification was hampered by the fact that the relatively short sequences obtained usually matched data in the GenBank database of a number of different families and genera. In a few cases, though, genera and even species could be assigned tentatively. Plant sequences from seven different orders, containing eight different families and genera were found in the dung. Especially at the order and family level, molecular results overlap considerably with identifications based on macrofossils. *Salix* appeared to be common, as in the macrofossil record. Several identifications could be narrowed down to genus level, especially in the Asteraceae (*Achillea*, *Bellis*, *Leucanthemum*). The identification of *Caltha palustris* agrees with the identification of *C. palustris* seeds. Although most, but not all, of the fossil determinations are not as detailed as the plant macrofossil identifications, these results show the potential value of fossil plant DNA analyses in arctic palaeoecology.

Several complete fruit-bodies of identifiable Ascomycetes were found inside the intestinal tract of the mammoth (Aptroot and van Geel, 2006), including fruit-bodies of dung-inhabiting Ascomycetes (*Sporormiella;* see Davis, 1987; Davis et al., 1977; Davis and Shafer, 2006). These Ascomycetes develop after at least one week of exposure to the air (Krug et al., 2004). The total absence of bile acids (fecal biomarkers; see below) is a strong indication that the ingested dung material was indeed mammoth dung. Dung of other species would have resulted in the detection of bile acids. Coprophagy, which has been repeatedly reported from herbivores (Fajardo and Hornicke, 1989), including elephants (Leggett, 2004) is suggested as a purposeful behaviour reported here for mammoths. Many herbivores are coprophagous in order to obtain certain vitamins. Rats prevented from eating their feces can suffer from deficiencies of vitamin K, complex B vitamins, biotin, and other vitamins (Fajardo and Hornicke, 1989).

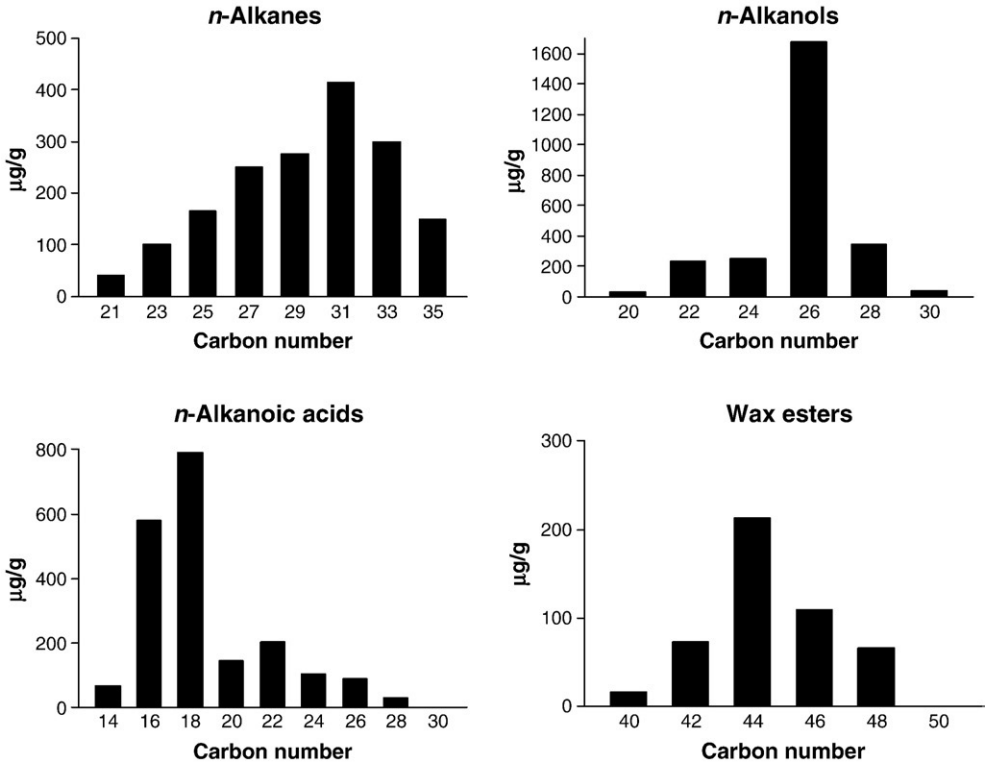

Figure 5. *n*-alkane, *n*-alkanol, *n*-alkanoic acid and wax ester distribution of the dung.

Earlier palynological studies, e.g. the study of the Jarkov Mammoth (Mol et al., 2006b; van Geel et al., 2007), have shown that spores of coprophilous fungi are common in the microfossil record of the 'mammoth steppe'. The presence of these spores is related to the wide availability of herbivore dung as a substrate. Coprophilous fungi played an important role in nutrient cycling in the steppe environment.

Chemistry

The dung consisted of 21.3% organic carbon, indicating that the main portion of the sample was composed of mineral particles. The total nitrogen content was 1.25%, resulting in a carbon to nitrogen ratio of 17.0, which implies well decomposed material (Heal et al., 1997).

Lipids

Extractable lipids derive from the epicuticular waxes in leaves as well as waxes from roots (Walton, 1990). Figure 5 shows the *n*-alkane, *n*-alkanol, *n*-alkanoic acid and wax ester distribution of the dung. Even-numbered alkanes and odd-numbered alkanols were virtually absent. Of the alkanoic acids only very small traces of C_{15} and C_{17} including *iso* and *anteiso* analogues were found (not shown), which are indicative of bacterial contributions (Saito, 1960; Akashi and Saito, 1960).

n-Alkanes of the dung maximized at the C_{31} homologue. *n*-alkanols were largely dominated by a C_{26} component, which is a typical alkanol signature of grass species (Walton, 1990; van Bergen et al., 1997). Alkanoic acids were dominated by C_{16} and C_{18} components. Often unsaturated C_{18} alkanoic acids occur in

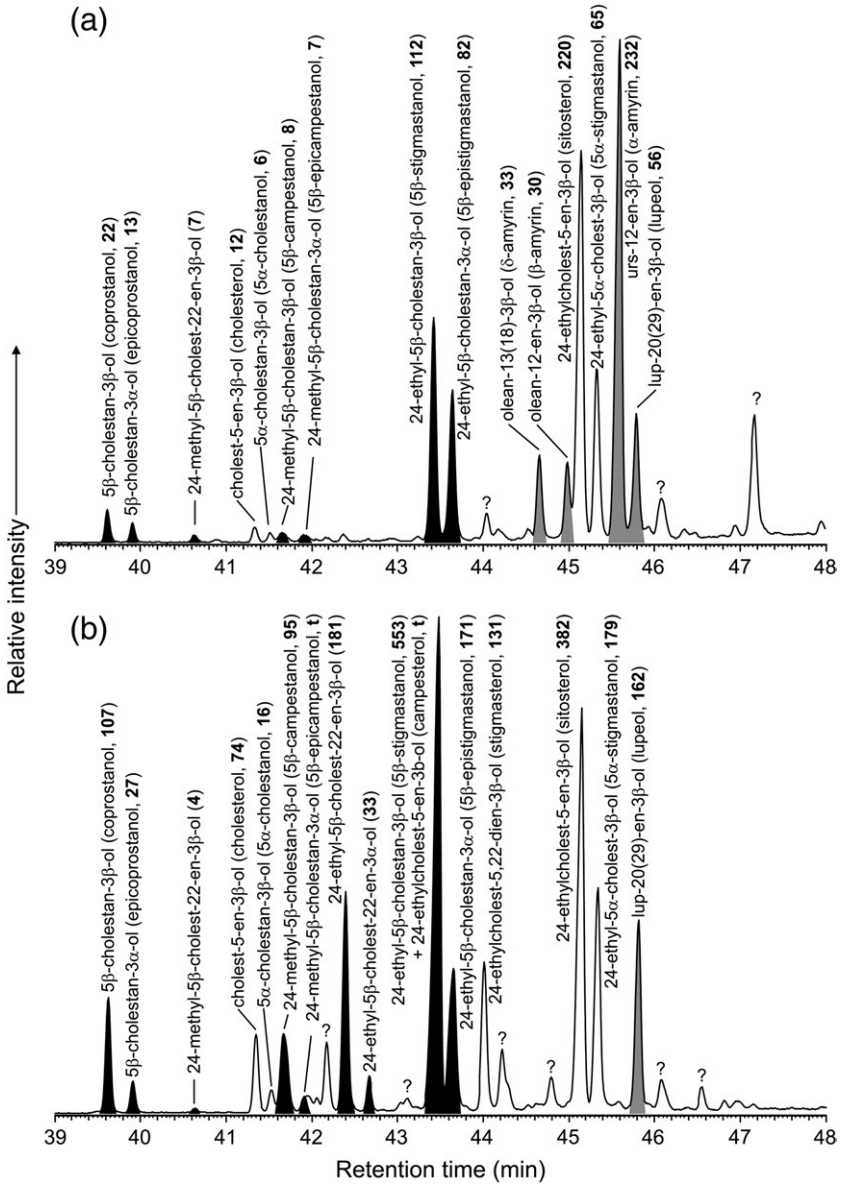

Figure 6. Partial gas chromatograms of sterol/triterpenol fractions isolated from (a) mammoth intestinal tract contents and (b) modern-day elephant dung. Peaks relating to fecal biomarkers are black while those corresponding to triterpenols are grey. Where appropriate trivial names are included in parentheses; bold values relate to concentration in units of μg g^{-1}dry weight.

plant-derived matter, but for the sample analysed these acids were hardly detected. Wax esters composed of an alkanoic acid and an alkanol moiety, were dominated by those combinations of which the alkanols had the same chain lengths as free alkanols. The alkanoic acids in the wax esters comprised mainly a C_{18} component. As a result, the most abundant wax ester, C_{44}, consisted predominantly of C_{26} alkanol and C_{18} alkanoic acid moieties. Altogether, the lipid composition reflects typical characteristics of higher plants (Walton, 1990, van Bergen et al., 1997) with hardly any contributions from other sources.

Fecal biomarkers

Figure 6a depicts the distribution of sterols and triterpenols obtained from the mammoth dung. For comparative purposes an analogous analysis of modern-day elephant dung was also made and the data obtained are presented in Figure 6b. While, unlike its modern equivalent, the mammoth dung does not contain a high abundance of Δ^{22}-5β-stanols (most likely these will have been lost through oxidative degradation) the data from the mammoth dung clearly show the presence of the more recalcitrant, saturated 5β-stanols. These compounds have been used previously as fecal biomarkers (Bethell et al., 1994; Bull et al., 1998, 1999b, 2002a, 2003), with a predominance of the C_{29} 5β,3β- and 5β,3α-homologues being consistent with the dung having derived from a herbivorous diet (Evershed et al., 1997; Bull et al., 1999a, 2002b). Moreover, the abundance (~ 294 $\mu g\ g^{-1}_{dryweight}$ cf. ~ 724 $\mu g\ g^{-1}_{dryweight}$ modern-day elephant dung) of these biomarkers attests to the remarkably good preservation exhibited by the intestinal tract contents and, at ca. 22,500 cal yr BP, this represents the oldest positive identification of fecal matter using organic geochemical methods.

Additional evidence for the herbaceous origin of the dung may be derived from the high abundance of pentacyclic triterpenoids (i.e. α-, β-, and δ-amyrin and lupeol), compounds predominantly derived from higher plant leaf waxes (Killops and Killops, 1993; van Bergen et al., 1997). These results and inferences may seem obvious in this context, but they are valuable because their context is certain. In future, the chemical fingerprints of mammoth feces can be of diagnostic value in

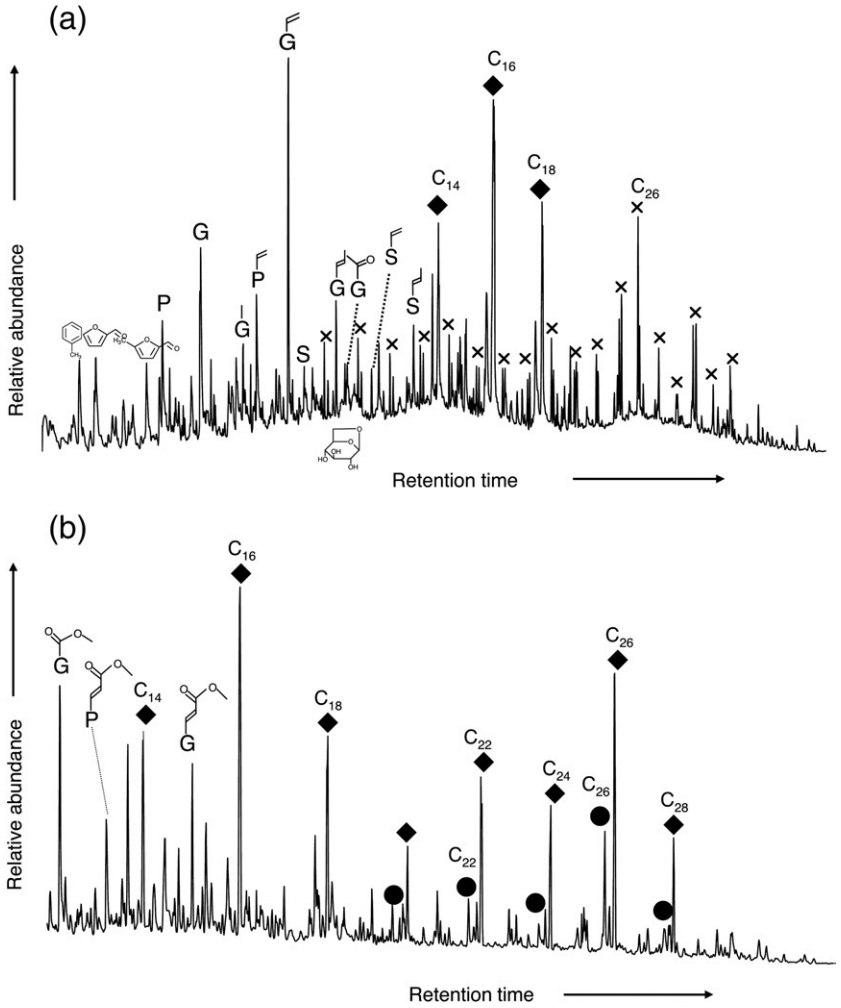

Figure 7. a: Partial gas chromatogram of the pyrolysate of the dung. Legend: P = phenol; G= guaiacol; S = syringol; side-chains of P, G and S are indicated; ×: doublet of *n*-alkene and *n*-alkane ● = 1-alkanol; ♦ = alkanoic acid; C_n indicates chain length. b: Partial gas chromatogram of the compounds released upon Thermally assisted Hydrolysis and Methylation. Legend: P = methoxybenzene; G = 1,2-dimethoxybenzene; ● = 1-methoxyalkane; ♦ = alkanoic acid, methyl ester; C_n indicates chain length.

determining the species in studies of permafrost (soil) material containing a dung component.

Bile acids represent another class of fecal biomarkers that may be used to assess the origin of material of a putative fecal origin (Elhmmali et al., 1997, 2000). Elephants, hyraxes and manatees are unique among mammals in that they do not produce any bile acids, this role being fulfilled by a suite of tetra- and pentahydroxylated bile alcohols (Kuroki et al., 1988; Hagey et al., 1993). Results obtained from the mammoth dung revealed a total absence of bile acids indicating that this is probably a physiological anomaly also shared by mammoths although further analyses to determine the presence of bile alcohols were not conducted at this stage. Whatever fecal matter the YM ate (fungal fruit-bodies present; see above), the absence of bile acids provides significant information: it was probably purposefully ingested mammoth dung.

Pyrolysis and thermally assisted hydrolysis and methylation (THM)

The pyrolysate (Fig. 7) of the dung sample was dominated by lignin-derived guaiacols and syringols. They were dominated by 4-vinylguaiacol, typical of grasses (van Bergen et al., 1998; Nierop, 2001). Other important lignin-derived compounds included guaiacol, 4-methylguaiacol, *trans* 4-(propen-2-yl)guaiacol, and their syringylic analogues along with 4-vinylsyringol.

Small amounts of polysaccharide-derived products were encountered. In addition, the same lipids identified after lipid extraction/analysis were found. Upon THM, methyl esters of alkanoic acids and methyl ethers of alkanols dominated along with methylated versions of vanillic acid, *p*-coumaric acid and ferulic acid. No ω-hydroxyalkanoic acids or α,ω-alkanedioic acids were identified. These compounds are present in cutin and suberin, which represent the protective surroundings of plant tissues (e.g. Kolattukudy, 2001). This absence suggests that cuticles largely disappeared after the digestion of the plant material. However, a homologous series of *n*-alkenes and *n*-alkanes (C_{10}–C_{31}) were identified in the pyrolysate of the dung and these products have been attributed to the aliphatic biopolymer cutan. This biopolymer has been found in the cuticles of some plants, but may also be the result of diagenetic processes during the 'fossilisation' (De Leeuw et al., 2006). This latter process may explain the absence of cutin monomers while stem fragments of grasses were still present.

Willow branches present in the dung exhibited a completely different picture (data not shown). They were dominated by a typical dicotyledon lignin signature, which consists of equal amounts of guaiacols and syringols. Along with the well preserved lignin, i.e. hardly any oxidation of side-chains, the high abundance of *p*-coumaric acid and ferulic acid shows that the majority of the dung consisted of grasses. Leaf tissues of willow were of minor importance. This is consistent with the results of the macrofossil analysis.

Discussion and conclusions

Reconstruction of the diet of extinct herbivores based on pollen and macrofossil remains in preserved gastrointestinal tracts can be problematic for several reasons. First of all, the proportion of easily identifiable plants such as mosses and leaves of shrubs and trees with indigestible ligneous parts is often overrepresented. Secondly, pollen may provide clues about surrounding vegetation but not necessarily record what the animal was eating. Thirdly, when animals take large mouthfuls of small-sized items they can also ingest unwanted secondary material. Fourthly, herbivores dying from starvation often do so with a stomach full of indigestible food that was desperately chewed on just before death, which gives a skewed picture of the actual diet. Finally, animals often shift from their staple, representative diet when transient resources are temporarily available (Guthrie, 1990).

Paleoecologists originally portrayed the unglaciated landmasses in Siberia as a productive arctic steppe (the 'mammoth steppe') which could support herds of large grazers such as the woolly mammoth, steppe bison, and horse, all relying heavily on grasses for their diet (e.g. Guthrie, 1990). Later studies challenged this concept as detailed fossil pollen identifications from lake sediments included many tundra species which suggested a paleoenvironment consisting of a sparsely covered herb tundra rather than a continuous grassland steppe (Cwynar and Ritchie, 1980; Cwynar, 1982; Goetcheus and Birks, 2001). These conflicting observations of limited vegetation cover with marginal productivity versus numerous fossil remains of a diverse and abundant grazing megafauna have been a topic of heated debate (e.g. Guthrie, 2001). To answer the question of how Siberia could support large mammals during a time of extensive glaciation and limited plant productivity, detailed reconstructions of paleoenvironments have been carried out in the last decade based on pollen and botanical macrofossils from sites that also contained Pleistocene mammal fossils.

The composition of the dung of the Yukagir mammoth indicates that this animal lived in a cold, treeless vegetation with a mosaic of perennial wet areas within a largely arid steppe-tundra. The presence of remnants of willow *(Salix*), marsh marigold (*Caltha*), sedges (*Carex*), rushes (*Juncus*) and *Drepanocladus. aduncus* in the dung which require permanent water availability attest to the presence of damp vegetation, perhaps with streams or standing water in summer and accumulated snow in winter. Fresh water was available for drinking, as shown by the presence of the green alga *Pediastrum* (Table 2). *Ranunculus nivalis* and *R. pygmaeus* are characteristic of snow-bed vegetation. Damp soils would have supported productive floodplain meadows with abundant grasses, sedges, and rushes, and tall herbs such as *Achillea*, *Petasites*, etc. (e.g. Zazula et al., 2006a). The fact that sage (*Artemisia*) and other perennial herbs such as sneezeweed (*Achillea*) and various meadow grasses (*Agrostis*, *Poa*) were present in the dung in large quantities suggests the mammoth was also grazing dry open grassland vegetation (steppe and arctic communities). Several of the herbs and the acrocarpous mosses occur in dry, open grasslands, such as *Potentilla hyparctica*, *Draba* spp., *Sagina/Minuartia*, *Papaver* sect. *Scapiflora, Polytrichum alpinum*, and also *Salix arctica*. The widespread arctic grassland and the willow vegetation provided enough fodder for large herbivores. Relatively little snow in the arid climate would mean that the dried grasses and willow shrubs were exposed and available to herbivores in winter. Disturbed areas allowed the

growth of weedy herbs such as *Rumex acetosella* and Chenopodiaceae spp. Grazing and trampling, combined with the cold, dry climate, resulted in open patches of soil where mineral dust was eroded and dispersed widely by wind (loess) over the landscape and its vegetation. The YM unavoidably ingested quantities of this mineral material with his food (see Fig. 4, 41, showing dust-incrusted moss). According to the model of Guthrie (2001), deposition of fine loess sediments may have been an important factor in the formation of productive soils and maintenance of open patches in the ground vegetation cover. Other factors would have been clear skies and associated aridity, which led to a deep thaw in summer and the development of extensive root systems that recycled nutrients. Especially the latter is considered crucial for early snow melt and late snow arrival which prolongs growing seasons and increases turnover of nutrients in the soil.

The exact cause of death of the mammoth cannot be determined directly from his remains. However, there is evidence for the season of death. Based on the observations that all the willow leaves were badly preserved and that no leaves were connected to twigs, we suggest that the Yukagir Mammoth died between two growing seasons — between late autumn and early spring. The bad preservation of the *Salix* leaves found and their minimal contribution to the meal as inferred by the results of pyrolysis may indicate that the YM consumed and partially digested dead dehisced leaves deposited in the hollow and exposed by melting snow. Any leaves that were still connected with twigs would have almost certainly been disconnected during this process. Detailed analysis of the annual rings of the dwarf willows shows that in the majority of the examined twigs the formation of the very first vessels in the terminal tree-ring could be detected, indicating that the twigs were browsed at the very beginning of the growing season (Fig. 3, 17). The well-developed leaf primordia indicate imminent new leaf expansion and the first fresh leaves that may have already expanded during the start of spring growth would have been soft and easily digested. The presence of seeds and fruits, particularly those of plants of late-lying snow habitats, in the meal support this inference, as they would also have been preserved over winter in a snow-filled hollow and revealed by spring melting. Death at the start of the growing season makes the Yukagir mammoth different from the Beresowka, Kirgilakh, Yuribey, Shandrin and Fishhook Mammoths, which all perished in late summer or autumn.

Significantly, the willow twigs, although broken, had not been squashed and the lignin was well preserved, suggesting a deficiency in the digestive process. The molars of the YM were still in place, but heavily worn. This could indicate that the old mammoth was unable to gain sufficient nourishment from his food. The additional stress of winter may have contributed to his demise. On the mammoth steppe, nutrient levels were low during the winter (Guthrie, 1990) and the start of the growing season might have come too late for this animal. Lying down in a sheltered hollow, he died, and became covered with a thick mud layer that slumped into the hollow during the warm season, which subsequently froze and preserved part of him.

Feeding habit and diet

Our analyses indicate that grasses and sedges were used as a dietary staple with herbs, shrubs and mosses as supplements and confirm previous assumptions that mammoths were grazers (Olivier, 1982). Because the Yukagir Mammoth died at a different time of year, a comparison of the plant remains of the YM with the other fossil mammoth remains adds insight into differences in the composition of the diet of the woolly mammoth through time and in space. The absence of tree remnants in the dung of the YM indicates a significant shift from the usual dietary pattern of mammoths. The pollen record of the dung indeed shows that trees were absent in the area where the YM died.

Twigs of alder (*Alnus*), birch (*Betula*), larch (*Larix*) and spruce (*Picea*) were found in all other mammoth dung analysed so far. Mammoths are generally assumed to have used the poor-quality fibrous resources of grasses as a dietary staple with occasional twig tips supplying nutrients that grasses lack (Guthrie, 1990). As woody plants are high in toxic compounds, it has always been assumed that cecal digesters, such as horses and mammoths, which lack a rumen for detoxification, could not consume too much of this type of plant material. Although the minor proportion of woody plants found in most mammoth dung samples analysed to date seems to support this assumption, the relatively high percentage of willow (*Salix*) in the dung of the Yukagir Mammoth (10–20% by volume) does not agree with this general strategy to avoid food poisoning. A possible explanation might be that the green parts of willow twigs are less toxic at the beginning of the growing season. It might be that they were an important food source for grazing mammoths in the spring. In a similar way, African elephants turn to browsing edible green parts of trees temporarily during the dry season (Guthrie, 2001). An alternative explanation might be that the Yukagir Mammoth was desperately eating willow because nothing else was available and this might

Table 5
Woolly mammoths found in Siberia with remains of dung in the gastrointestinal tract

Fossil	Lab. code/no.	^{14}C yr BP	cal yr BP	References
Yuribey mammoth	VSEGINGEO	9,600±300	10,192 – 11,978	Vasil'chuk et al. (1997)
Yukagir ammoth	see Table 1	ca.18,500 ca.	22,500	This study
Fishhook mammoth	Beta-148647	20,620±70	24,529 – 24,848	Mol et al. (2006b)
Shandrin mammoth	LU-595	40,350±880	n.p.	Vasil'chuk et al. (1997)
Kirgilakh mammoth	LU-718A	39,570±870	n.p.	Sulerzhitsky (1997)
Beresowka mammoth	T-299	31,750±2500	n.p.	Vasil'chuk et al. (1997)

Radiocarbon dates of woolly mammoths found in Siberia with remains of dung in the gastrointestinal tract. Calibration to calendar years (Stuiver and Reimer, 1993; Reimer et al., 2004) is not yet possible (n.p.) for samples older than 26,000 cal. yr BP (van der Plicht et al., 2004).

have led to a death caused by food poisoning. This seems less likely, though, as willow is an important spring browse of other herbivorous mammals such as white-tailed deer (*Odocoileus virginianus*), red deer (*Cervus elaphus*), elk and moose (*Alces alces*), muskox (*Ovibos moschatus*), arctic lemming (*Dicrostonyx torquatus*) and beaver (*Castor*) which are all unaffected by eating large quantities of willow leaves, twigs, and bark.

Climate and vegetation

The contents of dung samples of all mammoths analysed to date (Table 5) fit reconstructions of changing paleoenvironments through time as depicted in Guthrie (2006). During Pleniglacial interstadials, alder, birch, and pine could survive in Siberia and remnants of these trees have been found in the dung of the Beresowka, Kirgilakh, Shandrin, and Fishhook Mammoths. During the Last Glacial Maximum, treeless steppe vegetation existed in northern Siberia which explains why no remnants of trees were found in either the dung of the Yukagir Mammoth or in dung samples surrounding the carcass.

Global warming at the onset of the Late Glacial Interstadial resulted in the development of shrub and dwarf birch vegetation in northeastern Siberia, which was colonized by open woodland with birch and spruce during the Younger Dryas. Patches of closed larch and pine forests developed in the Holocene. The presence of remnants of larch and pine in the dung of the Yuribey Mammoth reflects the latter paleoenvironmental reconstruction (Table 5). Evidently, mammoths preferred to eat some woody material, but could obviously thrive on a herbaceous diet supplemented by dwarf willow twigs in areas of treeless vegetation.

Our results provide further evidence for the occurrence of a cold, treeless grassland vegetation during the last glacial maximum in northern Siberia more similar to steppe than tundra. We are confident that further integrated chemical, microscopic and molecular analyses of plant remains in dung of Beringian megafauna will eventually result in a more detailed reconstruction of this widespread glacial vegetation type and explain how mammoths could once have survived there.

Acknowledgments

D.D. and C.B. thank Siri CV, Hoofddorp, The Netherlands and its managing director Mr Jean-Philippe Reza for financial support and B.G. thanks the Fulbright Foundation for financial support. The authors thank the authorities of the Sakha Republic (Yakutia, Russia) for permission to study the dung of the Yukagir Mammoth. Jan van Arkel prepared pictures of microfossils and macro-remains.

References

Ager, T.A., 2003. Late Quaternary vegetation and climate history of the central Bering land bridge from St. Michael Island, western Alaska. Quaternary Research 60, 19–32.

Akashi, S., Saito, K., 1960. A branched saturated C15 acid (sarcinic acid) from sarcina phospholipids and a similar acid from several microbial lipids. Journal of Biochemistry 47, 222–229.

Andreev, A.A., Tarasov, P.E., Siegert, C., Ebel, T., Klimanov, V.A., Melles, M., Bobrov, A.A., Dereviagin, A.Yu., Lubinski, D.J., Hubberten, H.-W., 2003. Late Pleistocene and Holocene vegetation and climate on the northern Taymyr Peninsula, Arctic Russia. Boreas 32, 484–505.

Aptroot, A., van Geel, B., 2006. Fungi of the colon of the Yukagir Mammoth and from stratigraphically related permafrost samples. Review of Palaeobotany and Palynology 141, 225–230.

Bethell, P.H., Goad, L.J., Evershed, R.P., Ottaway, J., 1994. The study of molecular markers of human activity: The use of coprostanol in the soil as an indicator of human faecal material. Journal of Archaeological Science 21, 619–632.

Birks, H.H., 2001. Plant macrofossils. In: Smol, J.P., Birks, H.J.B., Last, W.M. (Eds.), Tracking environmental change using lake sediments. Volume 3, Terrestrial, algal and silicious indicators. Kluwer Academic Publishers, pp. 49–74.

Bull, I.D., van Bergen, P.F., Poulton, P.R., Evershed, R.P., 1998. Organic geochemical studies of soils from the Rothamsted Classical Experiments-II, Soils from the Hoosfield Spring Barley Experiment treated with different quantities of manure. Organic Geochemistry 28, 11–26.

Bull, I.D., Simpson, I.A., van Bergen, P.F., Evershed, R.P., 1999a. Muck 'n' molecules: Organic geochemical methods for detecting ancient manuring. Antiquity 73, 86–96.

Bull, I.D., Simpson, I.A., Dockrill, S.J., Evershed, R.P., 1999b. Organic geochemical evidence for the origin of ancient anthropogenic soil deposits at Tofts Ness, Sanday, Orkney. Organic Geochemistry 30, 535–556.

Bull, I.D., Betancourt, P.P., Evershed, R.P., 2002a. An organic geochemical investigation of the practice of manuring at a Minoan site on Pseira Island, Crete. Geoarchaeology 16, 223–242.

Bull, I.D., Lockheart, M.J., Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 2002b. The origin of faeces by means of biomarker detection. Environment International 27, 647–654.

Bull, I.D., Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 2003. Using steroidal biomarkers to track the abandonement of a Roman wastewater course at the Agora (Athens, Greece). Archaeometry 45, 149–161.

Challinor, J.M., 2001. Review: development and applications of thermally assisted hydrolysis and methylation reactions. Journal of Analytical and Applied Pyrolysis 61, 3–34.

Cooper, A., Poinar, H., 2001. Ancient DNA: do it right or not at all. Science 289, 1139.

Cwynar, L.C., 1982. A late-Quaternary vegetation history from Hanging Lake, Northern Yukon. Ecological Monographs 52, 1–24.

Cwynar, L., Ritchie, J.C., 1980. Arctic steppe-tundra: a Yukon perspective. Science 208, 1375–1377.

Davis, O.K., 1987. Spores of the dung fungus *Sporormiella*: increased abundance in historic sediments and before Pleistocene megafaunal extinction. Quaternary Research 28, 290–294.

Davis, O.K., Shafer, D.S., 2006. *Sporormiella* fungal spores, a palynological means of detecting herbivore density. Palaeogeography, Palaeoclimatology, Palaeoecology 237, 40–50.

Davis, O.K., Kolva, D.A., Mehringer, P.J., 1977. Pollen analysis of Wildcat Lake, Whitman County, Washington: the last 1000 years. Northwest Science 51, 13–30.

Davis, O.K., Mead, J.I., Martin, P.S., Agenbroad, L.D., 1985. Riparian plants were a major component of the diet of mammoths of Southern Utah. Current Research in the Pleistocene 2, 81–82.

De Leeuw, J.W., Versteegh, G.J.M., van Bergen, P.F., 2006. Biomacromolecules of algae and plants and their fossil analogues. Plant Ecology 182, 209–233.

Drescher-Schneider, R., Jacquat, C., Schoch, W., 2007. Palaeobotanical investigations at the mammoth site of Niederweningen (Kanton Zürich), Switzerland. Quaternary International 164–165, 113–129.

Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 1997. Bile acids as a new class of sewage pollution indicator. Environmental Science and Technology 31, 3663–3668.

Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 2000. Combined analysis of bile acids and sterols/stanols from riverine particulates to assess sewage discharges and other faecal sources. Environmental Science and Technology 34, 39–46.

Elias, S.A., Short, S.K., Nelson, C.H., Birks, H.H., 1996. Life and times of the Bering land bridge. Nature 382, 60–63.

Evershed, R.P., Bethell, P.H., Reynolds, P., Walsh, N.J., 1997. 5b-Stigmastanol and related 5b-stanols as biomarkers of manuring: analysis of modern

experimental materials and assessment of the archaeological potential. Journal of Archaeological Science 24, 485–495.
Fægri, K., Iversen, J., 1989. In: Fægri, K., Kaland, P.E., Krzywinski, K. (Eds.), Textbook of Pollen Analysis, 4th edition. Wiley and Sons, New York.
Fajardo, G., Hornicke, H., 1989. Problems in estimating the extent of coprophagy in the rat. British Journal of Nutrition 62, 551–561.
Fox-Dobbs, K., Leonard, J.A., Koch, P.L., in press. Pleistocene megafauna from eastern Beringia: paleoecological and paleoenvironmental interpretations of stable carbon and nitrogen isotope and radiocarbon records. Palaeogeography, Palaeoclimatology, Palaeoecology. doi:10.1016/j.palaeo.2007.12.011.
François, R.J., Eulderink, F., Bywaters, E.G., E.G.L., 1995. Commented glossary for rheumatic spinal diseases, based on pathology. Annals of the Rheumatic Diseases 54, 615–625.
Goetcheus, V.G., Birks, H.H., 2001. Full-glacial upland tundra vegetation preserved under tephra in the Beringia National Park, Seward Peninsula, Alaska. Quaternary Science Reviews 20, 135–147.
Gorlova, R.N., 1982a. Large remains of plants from the stomach of the Shandrin Mammoth. Trudy Zoologicheskogo Instituta 3, 34–35.
Gorlova, R.N., 1982b. The plant macroremains found in the enteron tract of the Yuribeyskiy mammoth. In: Sokolov, V.E. (Ed.), The Yuribeyskiy Mammoth. Nauka, Moscow, pp. 37–43.
Guthrie, R.D., 1990. Frozen Fauna of the Mammoth Steppe. The University of Chicago Press, Chicago.
Guthrie, R.D., 2001. Origin and causes of the mammoth steppe: a story of cloud cover, woolly mammal tooth pits, buckles, and inside-out Beringia. Quaternary Science Reviews 20, 549–574.
Guthrie, R.D., 2006. New carbon dates link climatic change with human colonization and Pleistocene extinctions. Nature 441, 207–209.
Hagey, L.R., Schteingart, C.D., Tonnu, H.T., Rossi, S.S., Hofmann, A.F., 1993. Unique bile alcohols (3,6,7,25,27-pentahydroxy cholestanes) and absence of bile-acids are a common feature of 3 ancient mammals. Hepatology 18 (4), A177–A177.
Heal, O.W., Anderson, J.M., Swift, M.J., 1997. Plant litter quality and decomposition: an historic overview. In: Cadish, G., Giller, K.E. (Eds.), Driven by Nature; Plant Litter Quality and Decomposition. CAB International, Wallingford, pp. 3–30.
Hofreiter, M., Poinar, H.N., Spaulding, W.G., Bauer, D., Martin, S., Possnert, G., Pääbo, S., 2000. A molecular analysis of ground sloth diet through the last glaciation. Molecular Ecology 9, 1975–1984.
Hofreiter, M., Betancourt, J.L., Sbriller, A.P., Markgraf, V., McDonald, H.G., 2003. Phylogeny, diet, and habitat of an extinct ground sloth from Cuchillo Cura, Neuquen Province, southwest Argentina. Quaternary Research 59, 364–378.
Iacumin, P., Davanzo, S., Nikolaev, V., 2006. Spatial and temporal variations in the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of mammoth hairs: palaeodiet and palaeoclimatic implications. Chemical Geology 231, 16–25.
Kienast, F., 2007. Plant macrofossil records — Arctic Eurasia. In: Elias, S.A. (Ed.), Encyclopedia of Quaternary Science vol. 3. Elsevier, pp. 2422–2434.
Kienast, F., Siegert, C., Derevyagin, A.Y., Mai, H.D., 2001. Climatic implications of late Quaternary plant macrofossil assemblages from the Taimyr Peninsula, Siberia. Global and Planetary Change 31, 265–281.
Kienast, F., Schirrmeister, L., Siegert, C., Tarasov, P., 2005. Palaeobotanical evidence for warm summers in the East Siberian Arctic during the last cold stage. Quaternary Research 63, 283–300.
Killops, S.D., Killops, J.K., 1993. An introduction to organic geochemistry. Longman Scientific and Technical. England, Harlow, Essex.
Kolattukudy, P.E., 2001. Polyesters in higher plants. In: Scheper, T. (Ed.), Advances in Biochemical Engineering/Biotechnology, vol. 71. Springer-Verlag, Heidelberg, pp. 1–49. Berlin.
Kompanje, E.J.O., 1999. Considerations on the comparative pathology of the vertebrae in Mysticeti and Odontoceti; evidence for the occurrence of discarthrosis, zygarthrosis, infectious spondylitis and spondyloarthritis. Zoölogische Mededeelingen Leiden 73, 99–130.
Kompanje, E.J.O., Klaver, P.S.J., De Vries, G.T., 2000. Spondyloarthropathy and osteoarthrosis in three Indomalayan bears: *Ursus ursinus* Cuvier, 1823, *Ursus thibetanus* Raffles, 1821, and *Ursus malayanus* Shaw & Nodder, 1791 (Mammalia; Carnivora: Ursidae). Contributions to Zoology 69, 259–269.
Krug, J.C., Benny, G.L., Keller, H.W., 2004. Coprophilous fungi In: Mueller, G.M., Bills, G.F., Foster, M.S. (Eds.), Biodiversity of fungi. Elsevier, Amsterdam, pp. 468–499.
Kuch, M., Rohland, N., Betancourt, J.L., Latorre, C., Steppan, S., Poinar, H.N., 2002. Molecular analysis of an 11,700-year old rodent midden from the Atacama Desert, Chile. Molecular Ecology 11, 913–924.
Kuroki, S., Schteingart, C.D., Hagey, L.R., Cohen, B.I., Mosbach, E.H., Rossi, S.S., Hofmann, A.F., Matoba, N., Une, M., Hoshita, T., Odell, D.K., 1988. Bile salts of the West Indian manatee, *Trichechus manatus latirostris*: novel bile alcohol sulfates and the absence of bile acids. Journal of Lipid Research 29, 509–522.
Leggett, K., 2004. Coprophagy and unusual thermoregulatory behaviour in desert-dwelling elephants of north-western Namibia. Pachyderm 36, 113–115.
Liu, T.S., Li, X.G., 1984. Mammoths in China. In: Klein, P.S., Klein, R.G. (Eds.), Quaternary Extinctions: A Prehistoric Revolution. University of Arizona Press, Tucson, pp. 517–527.
Mead, J.I., Agenbroad, L.D., Davis, O.K., Martin, P.S., 1986. Dung of *Mammuthus* in the arid southwest, North America. Quaternary Research 25, 121–127.
Mol, D., Tikhonov, A.N., van der Plicht, J., Bolshiyanov, D.Yu., 2003. Discoveries of woolly mammoth, *Mammuthus primigenius* (Proboscidea: Elephantidae) and some other Pleistocene mammals on the Taimyr Peninsula. Russian Journal of Theriology 2, 77–95.
Mol, D., Buigues, B., Tikhonov, A., Lazarev, P., van Geel, B., Fisher, D., Boeskorov, G., 2005. The Yukagir Mammoth — An Animal of the Cold Steppe (in Japanese). European Editions. Moscow.
Mol, D., Shoshani, J., Tikhonov, A., van Geel, B., Sano, S., Lazarev, P., Boeskorov, G., Agenbroad, L.D., 2006a. The Yukagir Mammoth: brief history, ^{14}C dates, individual age, gender, size, physical and environmental conditions and storage. Scientific Annals, School of Geology, Aristotle University of Thessaloniki, Special Volume 98, pp. 299–314.
Mol, D., Tikhonov, A., van der Plicht, J., Kahlke, R.D., Debruyne, R., van Geel, B., van Reenen, G., Pals, J.P., de Marliave, C., Reumer, J.W.F., 2006b. Results of the CERPOLEX/Mammuthus Expeditions on the Taimyr Peninsula, Arctic Siberia, Russian Federation. Quaternary International 142–143, 186–202.
Mol, D., Shoshani, J., Tikhonov, A.N., Boeskorov, G.G., Lazarev, P.A., Agenbroad, L., Suzuki, N., Buigues, B., 2007. Anatomical-morphological features and individual age of the Yukagir mammoth. In: Boeskorov, G.G., Tikhonov, A.N., Suzuki, N. (Eds.), The Yukagir Mammoth. Saint-Petersburg University Publishing House, Saint-Petersburg, pp. 97–118 (in Russian and in English).
Nierop, K.G.J., 2001. Temporal and vertical organic matter differentiation along a vegetation succession as revealed by pyrolysis and thermally assisted hydrolysis and methylation. Journal of Analytical and Applied Pyrolysis 61, 111–132.
Nierop, K.G.J., Verstraten, J.M., 2004. Rapid molecular assessment of the bioturbation extent in sandy soil horizons under pine using ester-bound lipids by on-line thermally assisted hydrolysis and methylation-gas chromatography/mass spectrometry. Rapid Communications in Mass Spectrometry 18, 1081–1088.
Nierop, K.G.J., Jansen, B., Hageman, J.A., Verstraten, J.M., 2006. The complementarity of extractable and ester-bound lipids in a soil profile under pine. Plant and Soil 286, 269–285.
Olivier, R.C.D., 1982. Ecology and behavior of living elephants: bases for adaptations concerning the extinct woolly mammoths. In: Hopkins, D.M., Matthews, J.V., Schweger, C.E., Young, S.B. (Eds.), Paleoecology of Beringia. Academic Press, New York, pp. 281–290.
Reimer, P.J., Baillie, M.G.L., Bard, E., Bayliss, A., Beck, J.W., Bertrand, C.J.H., Blackwell, P.G., Buck, C.E., Burr, G.S., Cutler, K.B., Damon, P.E., Edwards, R.L., Fairbanks, R.G., Friedrich, M., Guilderson, T.P., Hogg, A.G., Hughen, K.A., Kromer, B., McCormac, F.G., Manning, S.W., Ramsey, C.B., Reimer, R.W., Remmele, S., Southon, J.R., Stuiver, M., Talamo, S., Taylor, F.W., van der Plicht, J., Weyhenmeyer, C.E., 2004. IntCal04 Terrestrial radiocarbon age calibration, 26 – 0 ka BP. Radiocarbon 46, 1029–1058.
Rollo, F., Ubaldi, M., Erminin, L., Marota, I., 2002. Otzi's last meals: DNA analysis of the intestinal content of the Neolithic glacier mummy from the Alps. Proceedings of the National Academy of Science 99, 12594–12599.
Rothschild, B.M., Rothschild, C., 1994. No laughing matter: spondyloarthropathy and osteoarthritis in Hyaenidae. Journal of Zoo and Wildlife Medicine 25, 259–263.

Rountrey, A.N., Fisher, D.C., Vartanyan, S., Fox, D.L., 2007. Carbon and nitrogen isotope analyses of a juvenile woolly mammoth tusk: evidence of weaning. Quaternary International 169–70, 166–173.

Saito, K., 1960. Chromatographic studies on bacterial fatty acids. Journal of Biochemistry 47, 699–709.

Schmidt, N.M., Baittinger, C., Forchhammer, M.C., 2006. Reconstructing century-long snow regimes using estimates of high Arctic *Salix arctica* radial growth. Arctic, Antarctic, and Alpine Research 38, 257–262.

Sher, A.V., Kuzmina, S.A., Kuznetsova, T.V., Sulerzhitsky, L.D., 2005. New insights into the Weichselian environment and climate of the East Siberian Arctic, derived from fossil insects, plants, and mammals. Quaternary Science Reviews 24, 533–569.

Shoshani, J., Mol, D., 2007. Estimated shoulder height and weight of the Yukagir mammoth (*Mammuthus primigenius*). In: Boeskorov, G.G., Boeskorov, A.N., Suzuki, N. (Eds.), The Yukagir Mammoth. Saint-Petersburg University Publishing House, Saint-Petersburg, pp. 157–166 (in Russian and in English).

Solonevich, N.G., Tikhomirov, B.A., Ukraintseva, V.V., 1977. The preliminary results of the study of plant remnants from the enteron of Shandrin mammoth (Yakutia). Proceedings of the Zoological Institute Moscow 63, 277–280.

Stuiver, M., Reimer, P.J., 1993. Extended ^{14}C database and revised CALIB radiocarbon calibration program. Radiocarbon 35, 215–230.

Sulerzhitsky, L.D., 1997. The features of radiocarbon chronology of mammoths in Siberia and northern Eastern Europe (as substratum for human colonization). In: Velichko, A.A., Soffer, O. (Eds.), Humans Settle the Planet Earth: Global Dispersal of Hominids. Institute of Geography. Russian Academy of Sciences, Moscow, pp. 184–202 (in Russian).

Tütken, T., Furrer, H., Vennemann, T.W., 2007. Stable isotope compositions of mammoth teeth from Niederweningen, Switzerland: implications for the Late Pleistocene climate, environment, and diet. Quaternary International 164–65, 139–150.

Ukraintseva, V.V., 1979. Vegetation of warm intervals of late Pleistocene and the extinction of some large herbivorous mammals. Botanicheskii Zhurnal 64, 318–330.

Ukraintseva, V.A., 1993. Vegetation cover and environment of the Mammoth Epoch in Siberia. The Mammoth Site of Hot Springs. South Dakota Inc.

van Bergen, P.F., Bull, I.D., Poulton, P.R., Evershed, R.P., 1997. Organic geochemical studies of soils from the Rothamsted Classical Experiments — I. Total lipids, solvent insoluble residues and humic acids from Broadbalk Wilderness. Organic Geochemistry 26, 117–135.

van Bergen, P.F., Nott, C.J., Bull, I.D., Poulton, P.R., Evershed, R.P., 1998. Organic geochemical studies of soils from the Rothamsted Classical Experiments — IV. Preliminary results from a study of the effect of soil pH on organic matter decay. Organic Geochemistry 29, 1779–1795.

van der Plicht, J., Beck, J.W., Bard, E., Baillie, M.G.L., Blackwell, P.G., Buck, C.E., Friedrich, M., Guilderson, T.P., Hughen, K.A., Kromer, B., McCormac, F.G., Bronk Ramsey, C., Reimer, P.J., Reimer, R.W., Remmele, S., Richards, D.A., Southon, J.R., Stuiver, M., Weyhenmeyer, C.E., 2004. NOTCAL04: comparison/calibration ^{14}C records 26–50 cal kyr BP. Radiocarbon 46, 1225–1238.

van Geel, B., Aptroot, A., 2006. Fossil ascomycetes in Quaternary deposits. Nova Hedwigia 82, 313–329.

van Geel, B., Zazula, G.D., Schweger, C.E., 2007. Spores of coprophilous fungi from under the Dawson tephra (25,300 ^{14}C years BP), Yukon Territory, northwestern Canada. Palaeogeography, Palaeoclimatology, Palaeoecology 252, 481–485.

Vasil'chuk, Y., Punning, J.M., Vasil'chuk, A., 1997. Radiocarbon ages of mammoths in Northern Eurasia: implications for population development and Late Quaternary environment. Radiocarbon 39, 1–18.

Walton, T.J., 1990. Waxes, cutin and suberin. In: Harwood, J.L., Bowyer, J.R. (Eds.), Methods in Plant Biochemistry 4. Academic Press, London, pp. 105–158.

Wampler, T.P., 1999. Review — Introduction to pyrolysis–capillary gas chromatography. Journal of Chromatography A 842, 207–220.

Willerslev, E., Hansen, A.J., Binladen, J., Brand, T.B., Gilbert, T.P., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D.A., Cooper, A., 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. Science 300, 791–795.

Yurtsev, B.A., 2001. The Pleistocene "Tundra-Steppe" and the productivity paradox: the landscape approach. Quaternary Science Reviews 20, 165–174.

Zazula, G.D., Froese, D.G., Schweger, C.E., Mathewes, R.W., Beaudoin, A.B., Telkal, A.M., Harington, C.R., Westgate, J.A., 2003. Ice-age steppe vegetation in east Beringia. Nature 423, 603.

Zazula, G.D., Schweger, C.E., Beaudoin, A.B., McCourt, G.H., 2006a. Macrofossil and pollen evidence for full-glacial steppe within an ecological mosaic along the Bluefish River, eastern Beringia. Quaternary International 142–143, 2–19.

Zazula, G.D., Froese, D.G., Elias, S.A., Kuzmina, S., la Farge, C., Reyes, A.V., Sanborn, P.T., Schweger, C.E., Smith, C.A.S., Mathewes, R.W., 2006b. Vegetation buried under Dawson tephra (25,300 ^{14}C years BP) and locally diverse late Pleistocene paleoenvironments of Goldbottom Creek, Yukon, Canada. Palaeogeography, Palaeoclimatology, Palaeoecology 242, 253–286.