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A prehistoric Egyptian mummy: Evidence for an ‘embalming recipe’ and the evolution of early formative funerary treatments

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ABSTRACT

Interdisciplinary scientific investigations utilising chemical analysis, shotgun metagenomics, textile analysis and radiocarbon dating have been applied to the study of an intact prehistoric Egyptian mummy, allowing insights into when this individual lived and died, and the funerary treatments employed in the preparation of the body. Here we present the first evidence for an extant prehistoric mummy that has undergone treatment with notably similar formative complex ‘balms’ that would later constitute the classic embalming recipes employed at the height of pharaonic mummification some 2500 years later. Making the informed assumption that the provenance of the Turin body was Gebelein, Qena or Luxor (Thebes), the findings offer the first indication that this type of funerary recipe was likely to have been employed over a wider geographical area at a time when the concept of a pan-Egyptian identity was supposedly still developing.

1. Introduction

Mummy S. 293 (RCGE 16550) is the earliest preserved body in the Egyptian Museum in Turin (Bergamini, 1988, 33, pl. 31; Grilletto, 1988, 180, pl. 244; Grilletto, 1991, 11, 25; Museo Egizio, 2001, 27, Fig. 15; Vassilika, 2010, 7; Ugliano, 2015, Fig. 14). The body has been classified within the category of human remains traditionally known as ‘natural’ mummies, preserved through the action of the hot, dry desert sand (Grilletto, 1988, 1991; Museo Egizio, 2001). It has long been assumed that preservation of soft tissue in prehistoric bodies such as S. 293 was predominantly through the natural processes provided by the favorable burial environment, rather than by the deliberate physico-chemical

intervention that characterises the ‘true’ or ‘artificial’ mummification of later periods (e.g. Aufderheide, 2003, p. 253; Greenhill, 1705, p. 152; Ikram and Dodson, 1998, p. 108).

A recent interdisciplinary investigation of funerary textile wrappings from securely provenanced, Late Neolithic and Predynastic graves at Mostagedda in the Badari region of Upper Egypt (c. 4500–3350 BC) (see Fig. 1) has shown that contrary to common belief, prehistoric funerary wrappings were impregnated with complex mixtures of fats, resins and oils (Jones et al., 2014). These findings predate the previously accepted beginning of mummification by some 1500 years, thought to have begun in the Old Kingdom (the ‘pyramid age’), c. 2500 BC (David in: Nicholson and Shaw, 2000, p. 373).

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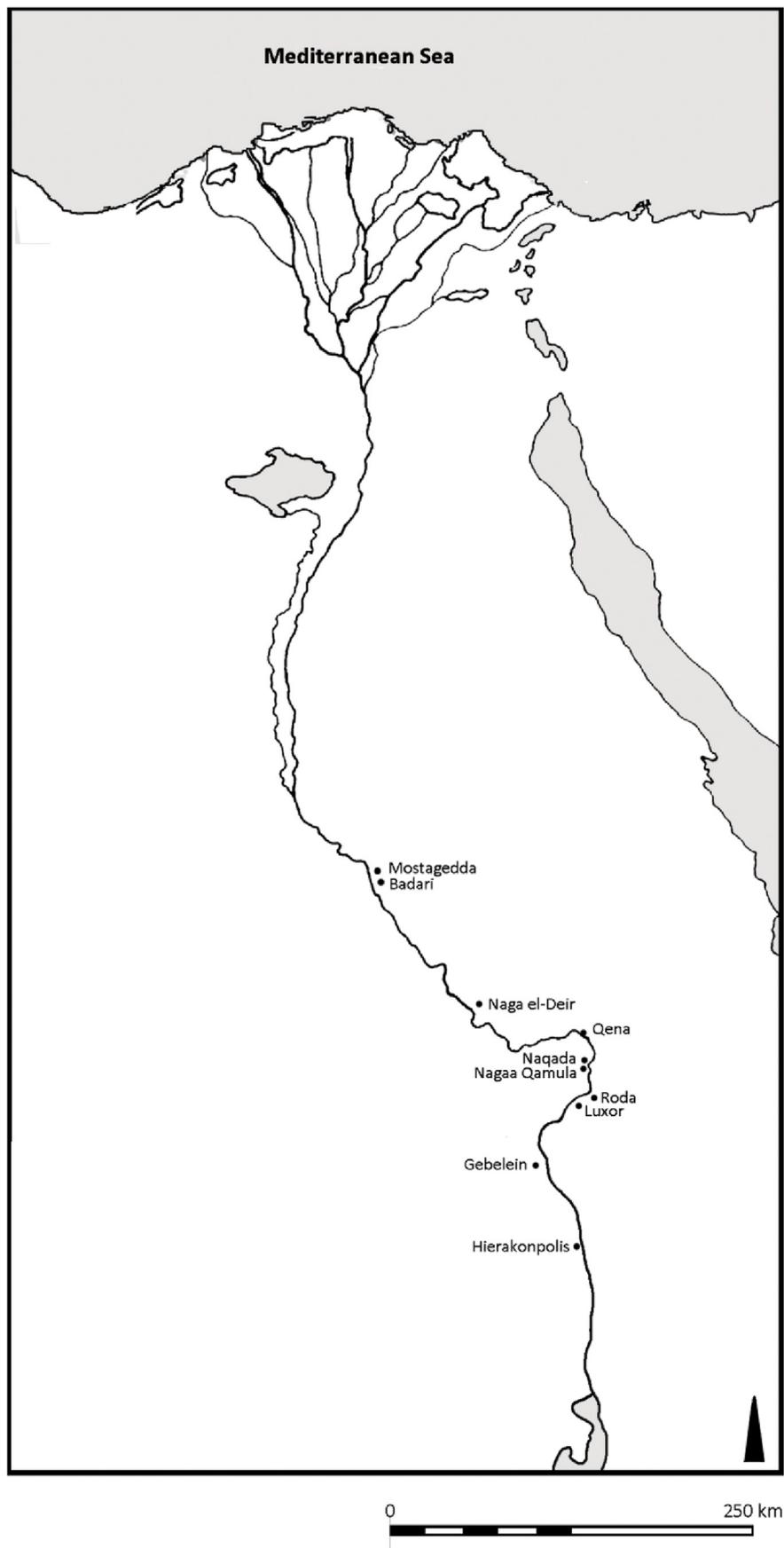


Fig. 1. A map of Egypt showing sites where preserved human remains wrapped in funerary textiles have been found (now in museums and referenced in the text).

Turin mummy S. 293 is one of a number of Egyptian prehistoric bodies in international museums (see Fig. 1). These include six bodies from Gebelein in Upper Egypt in the British Museum (Budge, 1920; Dawson and Gray, 1968; Taylor and Antoine, 2014), one on display in the University of Chicago Oriental Institute Museum from Naga el-Deir, another in the Field Museum in Chicago (Renee Friedman, pers. comm.), five bodies of unknown provenance in the Berlin Museum (Scharff, 1929) and an unprovenanced female in the Yale Peabody Museum of Natural History (ANTPA.000005. Maureen Daros, John Darnell, pers. comm.). Two bodies in the University of Pennsylvania Museum of Archaeology are possibly from a Predynastic cemetery at Nagaa Qamula, south of Naqada. One of these has undergone osteological and palaeopathological analysis, XRF analysis and radiocarbon dating (Hill and Rosado, 2017). A further three bodies in the Musée des Confluences from excavations at Roda and Gebelein (Lortet and Gaillard, 1909; Emmons et al., 2010, 51–53) have been radiocarbon dated, but no further analyses have been carried out (Quiles et al., 2014). The Gebelein bodies in the British Museum have been CT scanned (Taylor and Antoine, 2014; Antoine and Ambers, 2014), have undergone infrared imaging, isotopic analysis and have been radiocarbon dated (Friedman et al., 2018). No other analyses have been reported. Importantly, none of the studies used chemical analysis capable of characterising and identifying the presence of any funerary balms employed.

The majority of the bodies in the museum collections appear to have undergone either conservation treatments in the museums or some interference by the dealers from whom they were purchased, making valid scientific analyses problematic. Turin S. 293 does not appear to have undergone any such interventions, hence it provides a unique opportunity for analysis.

There are no written records of the provenance, date or circumstances of the discovery of Turin Mummy S. 293. The inventory of objects that were sent to the Turin Museum show that Ernesto Schiaparelli (1856–1928) purchased the body from an unnamed dealer in the early months of 1901, together with a collection of ‘prehistoric’ objects (S. 293–303, Turin State Archive, 1901). Entries indicate that Schiaparelli purchased some of the predynastic material in Luxor and also in Qena, where Tanios Girgis, an antiquities dealer, was known to deal in predynastic material from Gebelein (Quibell, 1901, p. 131).

Some of the objects were displayed with the body, such as textile fragments in a woven basket, a pair of plant fibre sandals, an ostrich skin bag and arrows (Museo Egizio, 2001) (Fig. 2). It is not clear



Fig. 2. Turin Mummy S. 293 (RCGE 16550). Dorsal view (Egyptian Museum, Turin).

whether any of these artefacts formed part of the original funerary ensemble. They have not been studied independently. The burial has been variously attributed to the Naqada II Period c. 3600/3500–3300 BC (Bergamini, 1988) and to the earlier Amratian Culture (Naqada I, c. 3700 BC) (Vassilika, 2010) with Gebelein as a possible provenance.

This individual has never been studied scientifically prior to our investigations; in particular, no surviving, intact prehistoric Egyptian mummies have been investigated specifically for evidence of ‘embalming agents’ and artificial preservation. Although the use of embalming agents in prehistoric burials as early as the late fifth millennium BC has now been established (Jones et al., 2014), this necessitated the analysis of funerary textiles from the Badarian and Naqada periods at the site of Mostagedda in Middle Egypt, with the bodies remaining in Egypt and unavailable for detailed scientific study. The Mostagedda textiles, like the samples from the Turin body, were studied microscopically, radiocarbon dated and subjected to chemical analysis. The Mostagedda samples included four dated to the Badarian period, one dating to the Naqada IIB phase and two dating to the Naqada IIC phase (broadly these Naqada period burials date to c. 3600 to 3350 BC, see Dee et al., 2013, 2014) (one could only be dated to the Naqada period more generally). Consequently, the date for the Turin body (Naqada IA to Naqada IIB; c. 3700–3500 BC) lies within the Badarian–Naqada period chronologies provided by the Mostagedda samples. We caution that there is some degree of overlap in the date estimates for the various phases of the Naqada period (Hendrickx, 2006; Dee et al., 2014) and debate regarding the question of whether they are in fact temporally ordered as previously assumed.

The organic extracts from the Mostagedda funerary textiles contained acyl lipids (fat/oil), conifer (pine) resin (all but one of the burials showed evidence of heating of this product), aromatic plant extracts, a sugar/gum and natural petroleum. The Badarian period samples also included constituents that indicated the possibility of a significant acyl lipid component originating from marine invertebrates (sponges). The research here provided the opportunity to investigate the possible presence of embalming recipes with preservative properties applied to the funerary textiles in direct contact with the body of an intact prehistoric mummy which had, like all other comparable existing examples, long been assumed to be a ‘natural mummy’.

2. Materials and methods

Here, we addressed a number of key questions by carrying out the following analyses on mummy S. 293:

- Visual inspection and collection of samples
- Microscopical analysis of 2 samples of textile, one each from the torso, to study the technology of the textiles
- Radiocarbon dating of 2 samples of textile from the same areas to date wrappings on the body and the textile fragments in the accompanying basket to ascertain whether they are contemporary
- Chemical analysis by gas chromatography-mass spectrometry (GC-MS) and thermal desorption/pyrolysis (TD/Py)-GC-MS of 3 samples of textile wrappings, one each from the torso, right wrist and accompanying basket of loose fragments, to allow the characterisation and identification of any organic natural products that may have been utilised as embalming agents
- Shotgun metagenomics to determine whether sequences from pathogens could be detected in DNA extracted from a fragment of skin.

2.1. Visual inspection and sample collection

The body lies on the left side in a contracted position (Fig. 2),

typical of the ‘foetal’ position observed in Egyptian prehistoric burials (Dawson and Gray, 1968; pls I, II). The overall condition of the body is very fragile. Radiographic investigations could not be performed to determine sex, age or evidence of any trauma because the body could not be moved from the display case without causing further damage. Previous reports suggest that the mummy is an adult male approximately 40 years old (Grilletto, 1988, p. 180). The genitalia are not visible. Subsequent macroscopical observation of visible teeth (right side) reveals that the upper first premolar (1.4), lower second premolar (4.5), first upper molar (1.6) and first lower molar (4.6) are still in situ. Upper and lower central and lateral incisors (1.1, 1.2, 4.1, 4.2) and upper and lower canines (1.3, 4.3) were lost post-mortem (Fédération Dentaire Internationale, 1971).

The cusp pattern on the first lower jaw premolar (4.6) shows a moderate degree of wear (Brothwell, 1989). Neither abscesses nor loss of bone and consequent resorption could be observed. This would imply that the person was in his second to third decade at time of death.

Fragments of linen textile remain attached to the back, shoulders, the right upper arm, the pelvic region, the lower limbs and the hands, suggesting that the entire body had been covered. Remnants of reed matting adhere to the lower limbs.

The skull is partially covered with patches of soft tissue, but the hair is not preserved. Portions of the sagittal and lambdoid sutures can be observed. The skin has become detached from the occipital bone and slipped to reveal the cranium underneath. An oval-shaped irregularity of the scalp, partly translating in the underlying bony region, is visible on the right parietal bone. This may be consistent with an intra-vitam lesion/trauma (i.e. an ossified skull fracture that occurred during life). (See Fig. S1, in SI).

There is post-mortem damage to the right shoulder. The right hand, with good soft tissue preservation, is disarticulated at the wrist. Textile is wound around the wrist and hand, forming a thick layer (Fig. S2, in SI). The left hand is skeletonised. The lower legs, feet and toe-nails are well-preserved.

Samples were collected in situ in the Egyptian Museum, Turin in May 2014. It was possible to take only a small number of samples without causing further damage to the mummy, while answering the questions posed in this study. A strict protocol for sampling was observed. Sterile instruments were used to avoid modern contamination and the samples were placed in sterile glass vials for analysis. The sample site, Museum and sample numbers were recorded.

2.2. Microscopical analysis of textiles

Two small samples of textile, one from the right side of the torso of S. 293, specimen TOR00011a 1 (0.5 cm × 0.7 cm, in two layers) and one from the basket, specimen TOR00013 3 (0.5 cm × 0.9 cm) were examined. Optical microscopy was employed for morphological examination and technical analysis to determine the general structure of the textiles. (For detailed methods see Jones, 2008, 102–105). Textile attributes, including the type and density of the weave and yarn construction, such as spin direction and diameter, were recorded based on guidelines by Centre International d’Etude des Textiles Anciens (CIETA, 1997), Walton and Eastwood (1988) and Emery (1995).

The specimens were photographed with a Zeiss Universal light microscope system equipped with transmitted light and epi-illumination optics. Darkfield epi-illumination techniques (illumination from above the specimen) with Zeiss HD (Hellfeld/Dunkelfeld) objectives and Olympus PM-10ADS photomicrographic attachments were employed (Oldfield, 1994; Rost and Oldfield, 2000). Kodak Elite Chrome 100 35 mm transparency film was used and selected images digitised (Figs. 3 and 4, below). The quality of the image at high magnification is currently superior to that which can be obtained with most digital cameras.

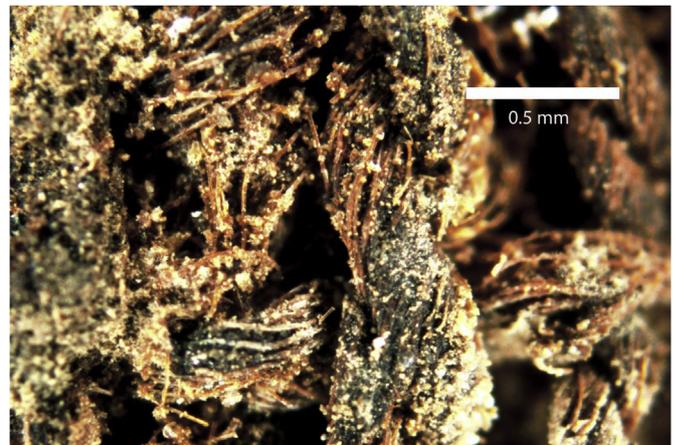


Fig. 3. Linen from body (middle of R. side of torso), side 1 of double layer. Epi-darkfield.



Fig. 4. Linen from basket, showing Z2S, a ‘prehistoric’ spin direction. Epi-darkfield.

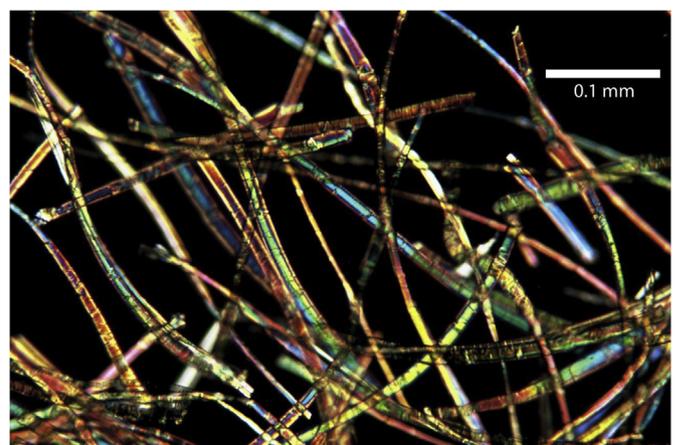


Fig. 5. Ultimate fibres, showing the typical features of flax, i.e. frequent cross markings, a small central lumen and low polarisation colours. Photographed in crossed polars, 50× magnification (Ron Oldfield). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

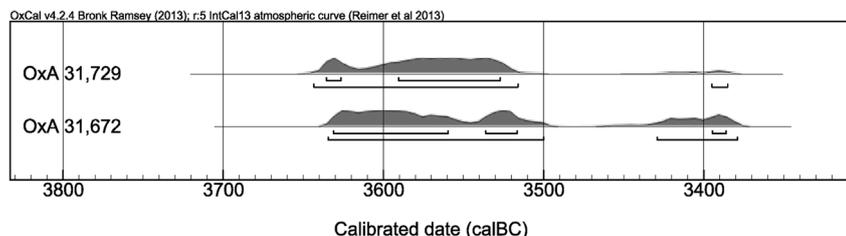


Fig. 6. Calibrated ages for the dated textile samples. The bars underneath the probability distributions are the 68.2% and 95.4% confidence intervals.

Separation of the yarn for identification of the fibre by electron microscopy was carried out under the stereomicroscope. A piece of yarn approx. 3 mm in length was excised from specimen TOR00013 3 from the basket and placed in a drop of glycerine as a mountant on a 3" x 1" microscope slide and eased apart with a pair of mounted needles. Specimen TOR00011a 1 from the torso was desiccated and heavily impregnated with a thick brown substance, making it impossible to extract intact fibres. Moreover, heavily impregnated fibres are opaque in transmitted light, masking their internal structure (Jones, 2008, 103).

Identification of the fibre was made using transmitted light illumination in crossed polars and comparing the image to modern fibre atlases (e.g. Catling and Grayson, 1982) (Fig. 5). For polarisation and other techniques of light microscopy see Oldfield, 1994. Measurements of the diameter of the ultimate (individual) fibres were made, using an eyepiece micrometer, calibrated for each magnification used.

2.3. Radiocarbon dating

Two samples of textile (~20–50 mg after treatment) were dated at the Oxford Radiocarbon Accelerator Unit, University of Oxford. One sample was from the body (torso) (TOR00011b 1) and the other from the basket (TOR00013b 3). The samples were rinsed with hexane (Fisher Scientific UK Distol; 40 °C, 2 h) prior to standard pretreatment. (For pretreatment methods, see Bronk Ramsey et al., 2004; Brock et al., 2010).

Radiocarbon ages were determined using the Oxford AMS (Bronk Ramsey et al., 2004) and are reported as conventional radiocarbon ages BP (Stuiver and Pollach, 1977) (Fig. 6). Calibrated radiocarbon ages against IntCal 13 (Reimer et al., 2013) were calculated using OxCal v4.2.4 (see Bronk Ramsey, 2009).

2.4. Chemical analysis

Gas chromatography-mass spectrometry (GC-MS) and sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) were employed to allow the characterisation and identification of free and bound/polymeric organic natural products utilised as embalming agents in the mummy studied (Buckley et al., 1999; Buckley and Evershed, 2001; Jones et al., 2014). The analytical approach reflects the assumed likely presence of a chemically-diverse range of biomarkers in these archaeological organic residues, with previous work on both prehistoric (Jones et al., 2014) and pharaonic (Buckley and Evershed, 2001; Buckley et al., 2004; Bianucci et al., 2015) impregnated funerary textiles in mind. Consequently, the analytical approach employed considered both this disparate biochemical nature and the likely prevalence of lipids in order to maximise the information on the organic residues extracted from the funerary textiles in this study. The textile samples analysed included: TOR00011a, from middle of right side of torso; TOR00013: from basket; TOR00014: from right wrist/hand. For methods, see Buckley et al., 1999; Buckley and Evershed, 2001; Jones et al., 2014.

2.5. Metagenomics

DNA was extracted from a skin fragment (0.5 cm × 0.2 cm) from the mummy and sequenced using shotgun metagenomics. (Shotgun metagenomics is the unbiased sequencing *en masse* of DNA extracted from a sample without target-specific amplification or capture (Kay et al., 2014, 2.)) Bioinformatic tools were used to map reads against a variety of pathogenic bacterial and parasitic reference genomes (including *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Brucella* spp, *Plasmodium falciparum*, *Leishmania donovani*, *Schistosoma haematobium*, *Toxoplasma gondii*). Metaphlan was also run for each sample to provide a 'snapshot' of which organisms were present in the sample. For methods, see Kay et al., 2014 and Kay et al., 2015.

3. Results and discussion

3.1. Microscopical analysis of textiles

3.1.1. Sample from torso TOR00011a 1

The condition of the brown, impregnated and heavily desiccated textile from the torso was poor. The weave was uneven, with a thread count of ~20 × 20 per square cm. The diameter of the yarn was ~0.4–0.5 mm. The yarns were spun in the Z2S direction (see below), but many yarns were flattened and unravelled, making it impossible to take accurate measurements (Fig. 3).

3.1.2. Sample from basket TOR00013 3

The condition of the sample from the basket was excellent, a natural golden brown colour with no visual evidence of significant impregnation either by resinous-looking substances or body fluids. The thread count was 20 × 20 per square cm. The diameter of the yarn was 0.4–1 mm. The yarns were spun in the Z2S direction, resulting in a loose, relatively even weave (Fig. 4).

The textiles from both the torso and the basket were woven of yarn first twisted in the 'Z' direction (i.e. to the right), and then doubled, or plied in the opposite, or 'S' direction (Z2S). This is characteristic of extant Egyptian textiles dating from the Fayum Neolithic (c. 5000 BC) up to Naqada IIB (c. 3500 BC), before a revolutionary change to single, 'S' spun yarns took place. This continued throughout the pharaonic period, resulting in the high standard of production of Egyptian pharaonic textiles (Jones and Oldfield, 2006, 33–35; Jones, 2008, 108–116). This is consistent with the radiocarbon data (see below).

Observation of ultimate (i.e. individual) fibres from TOR00013 3 in transmitted light illumination in crossed polars showed the typical features of flax (*Linum usitatissimum*), i.e. a fine central lumen or 'canal' and X-shaped cross markings at intervals along the length of the fibre (Fig. 5) (Catling and Grayson, 1982). The ultimate fibres were clean and well-prepared, fine to medium thickness, with a diameter of 8–18 µm with a mean of 16 µm, i.e. 0.16 mm.

3.2. Radiocarbon dating

Radiocarbon dating of two samples of textile, one from the body and one from the basket, gave a calendrical date range of 3635–3380 cal BC and 3644–3386 cal BC (at 95.4% probability). These dates are consistent with the earlier part of the Naqada period (Dee et al., 2013,

Table 1

Conventional radiocarbon and calibrated ages of textile samples. Calibrated ages are calculated using the IntCal 13 curve (Reimer et al., 2013) in OxCal v4.2.4 (Bronk Ramsey, 2009). $\delta^{13}\text{C}$ values are relative to VPDB and are expressed in parts per mille (‰).

| Sample | OxA | Conventional radiocarbon age | $\delta^{13}\text{C}/\text{‰}$ | Calibrated age BC (68.2% range) | Calibrated age BC (95.4% range) |
|-----------------|--------|------------------------------|--------------------------------|---------------------------------|---------------------------------|
| TOR0011b 1 S293 | 31,729 | 4778 ± 32 | −25.5 | 3636–3528 | 3644–3386 |
| TOR0013b 3 S293 | 31,672 | 4741 ± 30 | −25.2 | 3632–3387 | 3635–3380 |

2014). Uncalibrated conventional radiocarbon ages of the textiles and calibrated ages at 68.2% and 95.4% confidence intervals are displayed in Table 1 and Fig. 6. The radiocarbon dating is a significant addition to the chronology of the Late Neolithic and Predynastic Periods, which have been relatively poorly understood.

3.3. Chemical analysis

A combination of gas chromatography-mass spectrometry (GC-MS) and thermal desorption/pyrolysis (TD/Py)-GC-MS facilitated the molecular separation, characterisation and identification of both the free (solvent extractable) biomarker compounds, and the recognisable subunits of polymeric materials not amenable to the more conventional GC-MS approach (Buckley et al., 1999).

The amount of organic residue - having a resin-like appearance and often described as ‘resin’ historically - extracted from the three textile samples in this study constituted 5–11%, giving an average of 7%. This is in good agreement with prehistoric organic residues impregnating funerary textile samples analysed previously, giving 2–13% ancient ‘resin’, with an average of 7% (Jones et al., 2014). The abundance of the organic residues in the three textile samples associated with this mummy correlates with what would be expected for largely cellulose-based linen samples, providing a sound archaeological context for the chemical investigations.

The results of the chemical analyses are summarised in Table 2. The major products seen in all three extracts from the textile samples are degraded acyl lipids, derived from plant oils and animal fats (Table 2) and these dominated preliminary investigation where fatty acids were the main biomarkers observed. There is no evidence for the use of conservation treatments in the Museum archives and the very poor condition of the mummy would support this observation. Moreover, the outward appearance of the Turin mummy is not ‘shiny’, as are the British Museum Gebelein mummies, for example, which have undergone conservation treatments (Taylor, 2014). However, with an intact mummy such as S.293 it is sensible to consider the use of a siccative (drying) oil such as linseed which can be used in museum conservation treatments. Firstly, the relative distributions of the fatty acids observed are not typical of oxidised linseed oil (Fig. 7, S3, in SI). Specifically, the

palmitic/stearic (P/S) acid ratios are far greater than the ~1–2 typical of linseed oil (Mills and White, 1994; Serpico and White, 2000; Colombini et al. 2002, 2009; Bonaduce and Andreotti, 2009), or indeed human derived acyl lipids (Buckley et al., 1999; Buckley and Evershed, 2001). Additionally, the azelaic/palmitic ratios (A/P) are far less than the > 1 characteristic of a drying oil such as linseed (Colombini et al., 2002, 2009; Bonaduce and Andreotti, 2009). This cannot be explained by a co-mixing with a human source since human (and animal) adipose fats and skin lipids contain odd chain branched fatty acids (primarily $\text{C}_{15:0}$ and $\text{C}_{17:0}$) in small but significant quantities (Buckley et al., 1999; Buckley and Evershed, 2001). These branched fatty acids are highly resilient and are observed in archaeological samples deriving from mummified human tissue (Buckley et al., 1999; Buckley and Evershed, 2001). Consequently the complete absence of the branched fatty acids observed in human and animal adipose fats even at trace levels in the extracts, combined with an absence of squalene, an isoprenoid hydrocarbon abundant in skin lipids, argues against a significant human lipid component in the textile extracts and therefore negates the presence of linseed, even as an admixture with human lipids. It's also notable that oxalic acid is normally abundant in aged linseed oil (Colombini et al., 2002) and yet was absent from the sample extracts in this study, again indicating linseed oil was not used on this mummy.

Despite a lack of sterols associated with the source of the acyl lipids present, and accounting for processes of decay, the notably high palmitic to stearic acid ratios (Mills and White, 1994), combined with C_8 and C_9 homologues dominating the dicarboxylic fatty acids which derive from unsaturated acyl groups in the original fat/oil, suggest a non- or semi-drying plant oil high in oleic acid as the major constituent (Mills and White, 1994; Frankel, 1982). Notably, the dominance of a presumed plant oil in the extracted organic residue impregnating the funerary textiles is typical of a fat/oil being observed as the major ‘balm’ component in a previous study, where the proportion of animal fat/plant oil in the extracted organic residue impregnating prehistoric textiles ranged from 64 to 95% (Jones et al., 2014), compared to 96–97% for the samples taken directly from the Turin mummy. This is notably similar to previous studies on pharaonic embalming agents where the fat/oil component constituted 61–99% of the balms from pharaonic period mummies (Buckley and Evershed, 2001; Bianucci

Table 2

Egyptian Museum, Turin Predynastic/Naqada period mummy S. 293 Origin of balm samples and their chemical composition.

| Museum and Specimen No. | Historical date | Sample location and description | Inferred components of funerary balms | Relative abundance (%) ^a |
|-------------------------------|-------------------------------|------------------------------------|---------------------------------------|-------------------------------------|
| Male adult S.293 TOR00011a | Naqada IA-IIB ^b | linen from mid-right side of torso | Plant oil/animal fat | 96 |
| | | | Aromatic plant extract | 3 |
| | | | Sugar/gum | 0.5 |
| | | | Conifer resin | Trace |
| Male adult S.293 TOR00013 | Naqada IA-IIB ^b | linen from basket | Plant oil/animal fat | 100 |
| | | | Aromatic plant extract | 0.1 |
| | | | Sugar/gum | Trace |
| | | | Conifer resin | Trace |
| Male adult S.293 TOR00014 | Naqada IA-IIB ^b | linen from right wrist/hand | Plant oil/animal fat | 97 |
| | | | Aromatic plant extract | 2 |
| | | | Sugar/gum | Trace |
| | | | Conifer resin | 0.5 |

^a Percentage relative abundance of components in extracted ‘balms’ based on relative peak areas. Due to possible chemical changes over time, compositions do not imply that they were the original formulations.

^b Chronology based on radiocarbon dating of linen from torso and linen from basket (95.4% confidence) (Dee et al. 2013, 2014) and results of the textile analysis.

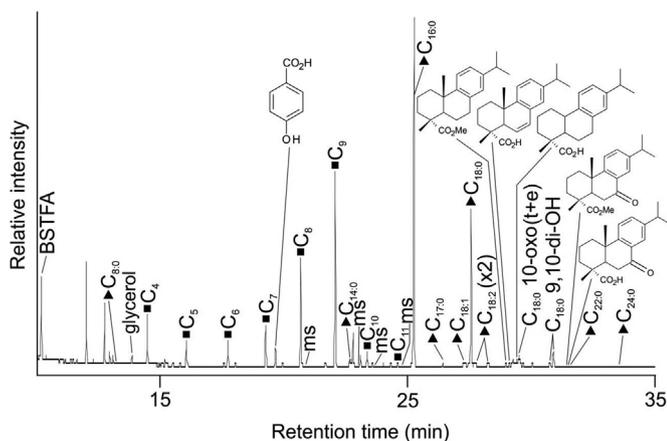


Fig. 7. Reconstructed gas chromatography mass spectrometry (GC-MS) total ion chromatogram (TIC) of the trimethylsilylated total lipid extract of Male Adult S.293 TOR0014, linen from right wrist/hand. Peak identities ('n' indicates carbon chain length; where shown, i indicates degree of unsaturation): filled triangles, $C_{n:i}$ indicates fatty acids; filled squares, C_n indicates α,ω -dicarboxylic acids; $C_{18:0}$ 10-oxo indicates a 10-oxo-octadecanoic acid; $C_{18:0}$ 9,10-di-OH (t + e) indicates 9,10-dihydroxyoctadecanoic acid (*threo* and *erythro* isomers). Also shown are glycerol, the structure of one aromatic acid: 4-hydroxybenzoic acid and five diterpenoids: methyl dehydroabietate, 6-dehydrodehydroabietic acid, dehydroabietic acid, methyl 7-oxodehydroabietate and 7-oxodehydroabietic acid; the letters ms represent monosaccharides.

et al., 2015).

Further chemical investigations focussing on the more minor constituents identified a conifer resin in the organic extract from the textile sample from the wrist, with the diterpenoid compound dehydroabietic acid clearly observed (Fig. 7) albeit as a minor component. It is commonly the major diterpenoid acid in aged conifer/pine resins (Pollard and Heron, 1996, p.247). Dehydroabietic acid was accompanied by several other diterpenoid biomarkers, identified by their relative retention times (dehydroabietic acid = 1.00) and mass spectra. These additional diterpenoid acids and their methyl esters were present as minor constituents of the conifer resin component and trace constituents of the organic residue/extract overall. In the sample from the wrist these include 7-oxodehydroabietic acid (RRT = 1.08; m/z 253 (bp), 268, 327 and 386 (M+)) as the major oxidised diterpenoid (~4.5% of the conifer diterpenoids detected) after dehydroabietic acid (~88%), along with 6-dehydrodehydroabietic acid (RRT = 0.99; m/z 237(bp), 355 ([M - 15]+) and 370 (M+)) (~2%), methyl dehydroabietic acid (RRT = 0.98; m/z 239(bp), 299 ([M - 15]+) and 314 (M+)) (~1.5%) and methyl 7-oxodehydroabietic acid (RRT = 1.07; m/z 253 (bp), 313 M - 15]+) and 328 (M+)) (~4%), which are typical diterpenoid biomarkers observed in archaeological samples containing conifer resin (e.g. Mills and White, 1997; Pollard and Heron, 1996; Colombini et al., 2000; Buckley and Evershed, 2001; Buckley et al., 2004; Colombini et al., 2005; Jones et al., 2014). Notably, the combined abundance of the methyl esters is indicative of processing of the conifer resin, since these are produced by the heating of the resin (Robinson et al., 1987; Beck et al., 1999; Hjulström et al., 2006; Jones et al., 2014), albeit the absence of retene and decarboxylated diterpenoids suggests any processing/heating was limited (Robinson et al., 1987; Beck et al., 1999; Hjulström et al., 2006; Jones et al., 2014), as does the presence of 7-oxodehydroabietic acid, rather than more highly oxidised species of diterpenoids (Mills and White, 1994). Importantly, both the relative abundance of the conifer resin in the 'balm' from the wrist/hand and the relative quantities of the oxidised and methylated diterpenoids in the resin component are entirely consistent with its application to the funerary textiles in antiquity from previous pharaonic (Buckley and Evershed, 2001; Bianucci et al., 2015) and prehistoric burials (Jones et al., 2014), confirming the presence of a conifer resin as

part of this embalming recipe. Unfortunately no potentially diagnostic monoterpenoids were identified and nor were the pimarane-type diterpenoid acids that might have allowed the determination of genus as has been possible previously (Jones et al., 2014).

The coniferous biomarkers methyl dehydroabietate and methyl 7-oxodehydroabietate were also identified, albeit as trace components, in the organic extract from the torso sample (Fig. S3, in SI). These again suggest possible heating since they are not the dominant diterpenoid compounds in unheated conifer resin (Hjulström et al., 2006), although their trace presence means any such interpretation remains somewhat tentative. The textile sample from the basket revealed an absence of any diterpenoid compounds even at trace levels (for potential significance see below). Despite the amount of conifer resin present in the 'embalming recipe' from the wrist of S. 293 being small (0.5%; Table 2) and the sample from the torso containing dehydroabietic acid as only a trace (< 0.1%) component, this is in agreement with the previous study on prehistoric funerary recipes (Jones et al., 2014) where five samples had only between 0.1 and 0.9% conifer resin in the 'balms'.

Aromatic acids characteristic of plant products were also present as minor constituents of the 'balms' in the two funerary textile samples applied to the body (2% and 3%; see Table 2 and Fig. 7, S3, in SI), with benzoic acid, 4-hydroxybenzoic acid and 4-hydroxy-3-methoxybenzoic (vanillic) acid being identified. These can be produced by the oxidation of lignin, which can be associated with flax (*Linum usitatissimum*), although it is minimal in the flax fibre itself. Notably, the hydroxyphenyl, guaiacyl and syringyl moieties characteristic of lignin (van der Hage et al., 1993; Galletti and Bocchini, 1995; Boerjan et al., 2003) were absent from the Py-GC-MS analyses, providing no clear biomolecular evidence for lignin, even at trace levels. This is consistent with the amount of total aromatics in linen fibres being extremely low (Morrison and Akin, 2001; Akin, 2013) and their absence in organic extracts from ancient linen textiles from the outer - 'clean' - layers employed in Egyptian mummification (Buckley and Evershed, 2001). In this context, the level of aromatic acids in the organic extracts from the funerary textiles applied to the mummy was an order of magnitude greater than might be expected if it were derived from degraded lignin (where one would also expect to observe syringic acid in the GC-MS, in addition to these two phenolic acids). In contrast, the phenolic acid (aromatic) component in the extract from the textile in the basket (0.1%) was not substantially more than what might be expected from the small amount of lignin present in the flax (for potential significance see below). The wide distribution of these phenolic acids in many plant-derived natural products make a precise identification of the source extremely difficult and would require further investigation. However, previous studies have revealed the presence of an 'aromatic plant extract' component (indicated by the aromatic/phenolic acids identified) in 'balms' identified within funerary textiles used to wrap the bodies and resinous-looking materials applied to those bodies in both prehistoric burials and Pharaonic and Graeco-Roman mummies (e.g. Mejanelle et al., 1997; Buckley and Evershed, 2001; Buckley et al., 2004; Jones et al., 2014; Bianucci et al., 2015). The 2–3% observed for the 'balsam'/aromatic plant extract in the textile 'balms' applied directly to the body (Table 2) is comparable to those in previously investigated Badarian and Predynastic/Naqada Period textiles (2.4–20%; Jones et al., 2014) and later pharaonic balms (trace–16%; Buckley and Evershed, 2001).

The chemical evidence for monosaccharides in the solvent extracts of the textile samples analysed suggest the possibility of a plant gum or sugar being a component of the 'balms'. In the extract from the torso, which contained the highest abundance of gum/sugar biomarkers (see Table 2 and Fig. S3, in SI), five pyranose (e.g. glucose; base peak m/z 204) and one furanose (e.g. fructose; base peak m/z 217) monosaccharides were detected. Similarly, the extract from the wrist revealed three pyranose and one furanose monosaccharides, albeit forming only a trace component of the 'balm'. Notably, however, these are the same sugar/carbohydrate markers observed in the sample extract from the torso, with the other two pyranoses likely to be below the

detection limits of the analysis in the case of the extract from the wrist. A furanose monosaccharide was tentatively identified in the sample from the basket, indicating a possible trace plant gum/sugar component, although it is not possible to establish a possible source in this case. The similarity of the relatively complex chemical profile of the carbohydrate markers in the torso and wrist sample extracts, in contrast to the very different profile for the basket sample extract, combined with the presence of both pyranose and furanose monosaccharides, argues against the gum/sugar component in these samples deriving from the cellulose-based linen wrappings (Buckley et al., 2004; Jones et al., 2014). Further work, focussed specifically on the sugar/gum component, would be needed in order to identify the possible source. Although very minor in abundance here, the sugar/gum component was observed in similarly very small proportions (trace-0.5%) as has been observed in a previous study of prehistoric funerary textiles, skin and ‘resinous’ material (0.1–4%; Jones et al., 2014) and later pharaonic balms (0.3%; Buckley and Evershed, 2001).

The thermal desorption-GC-MS total ion chromatograms (TIC) for all three samples (see SI) revealed carbon dioxide as the main component observed in the torso and wrist samples from the mummy. These findings are consistent with the nature of the sample (textile) and results of the GC-MS analysis. The presence of carbon dioxide in the TD profile again suggests a highly oxidised and degraded textile, particularly those samples from the mummy itself, since this is not observed in significantly later pharaonic textiles, where only pyrolysis at 610 °C generates this gas (Buckley and Evershed, 2001). The pyrolysate TICs for all three samples (see SI) revealed carbon dioxide as the main component observed, being more dominant in the samples from the mummy and far greater in abundance than that observed in the TD-GC-MS. Its dominance indicates highly oxidised samples and suggests the two textile samples from the body, in particular, are very highly degraded. The furan and pyran derivatives observed in the pyrograms of cellulose-based linen wrappings from later pharaonic times were absent (Buckley and Evershed, 2001), also confirming the highly degraded nature of the textiles.

The chemical analyses reveal that the original plant oil has become oxidised, partially polymerised and carbonised, which would explain the superficial resemblance to ‘resin’ observed on the funerary textiles from the torso and right wrist/hand.

The results of the chemical investigations reveal a mixture of several components constituting the ‘embalming resin’ impregnating the textiles applied to the body and somewhat similar to previous prehistoric recipes observed (Jones et al., 2014). Although there are some similarities with the organic residues extracted from the three textile samples, the absence of conifer resin in the organic extract from the textile in the basket, combined with the relatively very minor aromatic component, may suggest it wasn't in direct contact with the body within the burial assemblage.

Most notable perhaps is the clear presence of a conifer resin in the textile sample from the wrist, in contrast to its presence only as a trace constituent in the textile sample from the torso. This may reflect the reported early use of layers of linen impregnated with a ‘resinous’ substance wrapped around the back of the head, jaw and hands in particular at Hierakonpolis, Cemetery HK43. These observations were based on physical appearance only. Biochemical analyses have not been carried out, hence the nature and composition of these residues at Hierakonpolis remain unknown (Friedman et al., 2002; Jones, 2007, 980–982).

The results of the analysis of the ‘balm’ impregnating the textiles revealed a complex mixture. The recipe consists of a plant oil ‘base’ constituting the bulk of the ‘balm’, with far lesser amounts of a conifer resin, an aromatic plant extract/‘balsam’ and a plant gum/sugar. These relative abundances are typical of both those used in the prehistoric period (Jones et al., 2014) and those utilised in mummification throughout much of ancient Egypt's 3000-year pharaonic history (Buckley and Evershed, 2001). Importantly, there was biomolecular

evidence for processing of at least one of the natural components of this ‘balm’, i.e. the conifer resin, although both the low abundance and particular chemical nature (only methyl esters, no retene or other de-functionalised diterpenoids) of the compounds suggests only very moderate heating when compared to the evidence for processing observed in the Mostagedda ‘balms’ (Jones et al., 2014).

Significantly, there was no evidence for the natural petroleum product that was present in the majority of samples from Mostagedda, including the near contemporary Naqada IIB (c. 3500 BC) example. Although it is difficult to extrapolate from only one individual from Upper Egypt, it may reflect the greater geographical distance between Naqada period sites in Upper Egypt (e.g. Gebelein) and the nearest natural petroleum sources (around the Gulf of Suez and further north), when compared to the site of Mostagedda in Middle Egypt. Interestingly, there was also a lack of evidence for the unusual and abundant marine invertebrate biomarkers, posited to derive from a marine invertebrate source and reported in the ‘balms’ of primarily Badarian period funerary textiles (Jones et al., 2014). This is in agreement with the dating of this mummy which puts it a little after the Badarian-Naqada cultural transition when a change from seasonal pastoralism, with known connections to the Red Sea, to an increasing dependence on agriculture and a more sedentary existence along the Nile Valley was taking place. Notably, these recipes contain materials with antibacterial properties and in the case of the non-native conifer resin would have been imported from the eastern Mediterranean, providing evidence for long distance trade routes between Upper Egypt and the eastern Mediterranean at this time.

Taken together, the results of this study and those from the Mostagedda material indicate that significant standardisation in the constituents used in these formative funerary ‘balms’ may have already been developing during the Naqada period, albeit perhaps also with some regional variations at a time of ‘cultural flux’ in prehistoric Egypt. However, despite these important and interesting regional cultural nuances, deserving and needing further investigation, we present here the first example of an extant Egyptian mummy to display the utilisation of prehistoric recipes that would constitute one of the vital processes for the preservation of Egypt's most iconic New Kingdom mummies, beginning some 2000 years later.

3.4. Metagenomics

No DNA sequences derived from pathogens were detected by metagenomics in a sample of skin. This may reflect the fact that the individual was not infected at the time of death or that skin is not a suitable sample for detection of most infections. However, even if pathogen DNA were present in the sample at the time of death, it is likely to have been degraded over the millennia. Until recently, the Museum did not have temperature and humidity controls. The body had been exposed to high temperatures (up to 32 °C) and high humidity over the past 100 years, as well as during the five and a half thousand years of interment.

4. Conclusions

This study presents the first extant Predynastic mummy (dating to Naqada IA-IIB; c. 3700–3500 BC) with unequivocal scientific evidence for ‘embalming agents’ employed in the funerary treatment of the body. The results reveal a recipe of a plant oil, with far lesser amounts of a heated conifer resin, an aromatic plant extract/‘balsam’ and a plant gum/sugar, which is notably similar to those ‘balms’ identified in prehistoric times both in terms of the constituents and relative proportions of those ingredients. Moreover, this recipe contained antibacterial agents, used in similar proportions to those employed by the Egyptian embalmers when their skill was at its peak, some 2500 years later. Assuming the Turin mummy originates from the area between Naga el-Deir and Gebelein, based on all other known examples, the findings

provide first evidence to suggest the use of a similar complex prehistoric funerary recipe was likely to have been extended over a wider geographical area, albeit with some variation possibly reflecting regional differences.

Consequently, the antibacterial properties of the resin and ‘balsam’ ingredients and the similarity of the prehistoric recipe to those embalming agents utilised at the zenith of ancient Egyptian mummification provide further evidence for the early use of embalming substances affording localised soft tissue preservation and as such represent here the literal embodiment of the antecedents of classic mummification, which would become a central tenet of ancient Egyptian culture.

Author contributions

Conceived and designed the experiments: SAB JJ TFGH RB MJP. Performed the experiments: SAB JJ DC RB GK RO. Analysed the data: SAB JJ DC TFGH RB GK MJP RO. Contributed reagents/materials/analysis tools: SAB TFGH RO MP. Contributed to the writing of the manuscript: SAB JJ DC TFGH RB GK MJP RO FU.

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Appendix A. Supplementary data

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