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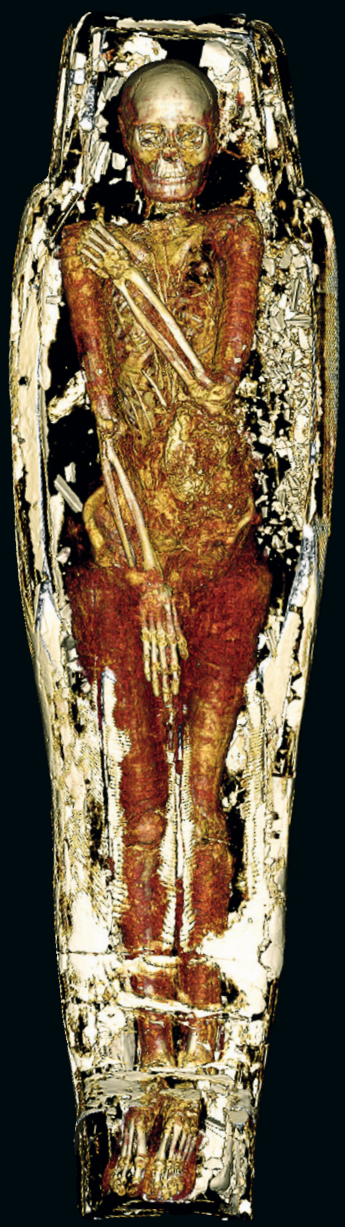

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Molecular Confirmation of *Schistosoma* and Family Relationship in two Ancient Egyptian Mummies

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Abstract

Egg morphology and immunocytochemistry have identified schistosomiasis in ancient Egypt. Our study aimed to detect and characterize schistosomal DNA in mummified human tissue. Liver samples from the mummy Nekht-Ankh (c. 3900 BP) and intestinal samples from Khnum-Nakht, possibly his brother, were analyzed using PCR primers suitable for fragmented ancient DNA, specific for either *Schistosoma mansoni* or *Schistosoma haematobium*. Mitochondrial primers examined any relationship between the supposed brothers. Two independent laboratories confirmed *S. mansoni* DNA from the Nekht-Ankh liver. One laboratory detected *S. haematobium* DNA in both the Nekht-Ankh liver and intestinal samples from Khnum-Nakht in repeat experiments. We believe this is the first verified report of *S. mansoni* in ancient Egypt. Although no *S. haematobium* DNA sequence was obtained, the results support earlier histological findings of *S. haematobium* in ancient Egyptian mummies. These findings demonstrate that both *S. mansoni* and *S. haematobium* were present in Central Egypt during the Middle Kingdom, around 3900 years ago. From the mitochondrial DNA analysis it appears that these two individuals were not maternally related, which is consistent with the morphology of the skulls. The lack of genetic relatedness between these supposed brothers throws light upon the habit of adoption in this society.

Introduction

Schistosomiasis has been extensively documented in Egypt, and *Schistosoma* eggs were detected in a pre-Dynastic mummy from 3200 BC (Deelder et al 1990). Today around two billion people are infected in the world, with 300 million suffering severely from the disease. Egypt continues to be a major affected area and in 2009 there were 820 442 treated cases reported (WHO 2011). Descriptions of medical conditions consistent with schistosomiasis have been documented in the Egyptological literature (Adamson 1976, Jordan 2000, Nunn & Tapp 2000). Direct evidence of schistosomiasis was obtained when a paleopathologist found the calcified eggs of *Schistosoma haematobium*, recognized by their morphology, in preserved kidneys from two mummies of the Twentieth Dynasty (Ruffer 1910). Further morphological evidence of schistosomiasis was obtained from a 5000 year-old mummy (Deelder et al. 1990), the bladder of a New Kingdom mummy dating to c. 3450 BP (Horne 2003) and a 65 % prevalence found among mummies (350–550 CE) from the Wadi Halfa area on the Sudan-Egyptian border (Miller et al. 1992). In the 1990s, a major study of schistosomiasis in ancient Egyptian mummified remains was undertaken at the University of Manchester (Contis & David 1996). Immunocytochemistry was pioneered as a diagnostic tool to detect

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Fig. 1. Anthropoid coffins of Nekht-Ankh (right) and Khnum-Nakht (left) as displayed in the Manchester Museum, inventory numbers 11724 and 11725, respectively. According to the coffin inscriptions, both individuals were sons of the same mother, who was called Khnum-Aa and carried the title "Mistress of the House", indicating she was an heiress who owned property. The inscriptions do not make it clear if both brothers shared the same father. Nekht-Ankh and Khnum-Nakht were buried together without wives or offspring, suggesting that neither man was married (David 2003).

the disease (Rutherford in David 2008: 99–115), followed by an enzyme-linked immunosorbent assay (ELISA) and DNA as confirmatory tests (Rutherford in David 2008: 116–132).

Detection and characterization of *Schistosoma* infection in ancient human remains may help us understand the relationship between the human host and parasite in the context of activities and environmental surroundings. PCR may be more sensitive than immunological methods. Therefore, the present study was undertaken to determine whether mummified remains contain schistosomal DNA and, if so, which species are present.

Until recently, PCR received relatively little attention in clinical diagnosis of schistosomiasis, as non-molecular methods are more cost-effective in countries with a high incidence of the disease. The *Schistosoma* genome project increased the number and range of available nucleotide sequences, facilitating the differentiation of the major human *Schistosoma* pathogens by PCR (Hamburger et al. 2001, Kane & Rollinson 1998, Pontes et al. 2002). Today the total genome sequence of *Schistosoma mansoni* is known (Berriman et al. 2009), but that of *Schistosoma haematobium* was determined only after the completion of this study (Young et al. 2012). In archaeological material often only small fragments of DNA (<150 bp) survive, although this varies greatly according to the local environmental conditions at the particular archaeological site (Pääbo 1989). Therefore, the first aim of the present study was to design and verify species-specific primers based on a short target sequence. Thereafter, tissues were examined for *Schistosoma* from two individuals believed to be brothers, dating from 3900 years ago (Fig. 1) and located in the Manchester Museum: Nekht-Ankh (inventory no. 11724) and Khnum-Ankh (inventory no. 11725). Further details can be found at: http://www.ancient-egypt.co.uk/manchester/pages/the%20two%20brothers.htm

The bodies of the "two brothers" have been subjected to various studies. In 1908 they were unwrapped and autopsied together with a multidisciplinary team by Dr. Margaret Murray, then curator of the Egyptian collection at the Manchester Museum (Fig. 4). This pioneering investigation marked a critical stage in the development of paleopathology. There was no opportunity to examine the bodies using radiological or histological techniques, but, relying on the use of morbid anatomy, the team made several interesting disease identifications (David 2003). In this original investigation it was noted that while both bodies were poorly preserved the color and texture of the residual tissue appeared very different in each case. Therefore, it was concluded that different preservatives had been used in the mummification process. Since 1973 the now skeletal remains of the "two brothers" have been the subject of continuing investigations by the Manchester Egyptian Mummy Research Project; techniques have included radiography, histology, electron microscopy, immunocytochemistry and paleoodontology (David 1979, 2000). Studies on the fragments of lung tissue indicated the presence of sand pneumoconiosis; when the ancient embalmers eviscerated the thoracic cavity they also removed part of the wall of the heart, which was attached to the lung. Here the histological sections

show fibrous tissue obliterating the pericardial and pleural cavities, indicating some time before death there must have been inflammation in this area, possibly associated with pneumonia. In well-preserved liver tissue a section containing a group of cells with thickened walls was tentatively identified as the remains of the liver fluke, *Facciola hepatica*.

A sample of the liver from the Egyptian mummy Nekht-Ankh and a sample of intestine from Khnum-Nakht, believed to be his brother, were analyzed by PCR for schistosomal DNA. Their family connection remains controversial, so to try to resolve the matter, primers for human mitochondrial DNA were used to determine any maternal relationship.

Materials and methods

Precautions against contamination

The standard strict precautions were taken at all stages of the study to ensure there was no cross-contamination (Donoghue 2008). Negative DNA extraction and PCR controls were always included in the protocols. All initial work on DNA extraction and PCR optimization was completed more than 12 months before the ancient DNA investigation and thorough cleaning had been undertaken in the interim.

Material for optimization and verification experiments

The Kuvin Centre, Jerusalem (Laboratory 1) supplied purified DNA for optimization and verification of experimental PCR primers. *Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma intercalatum* were supplied as adult pairs of worms, preserved in ethanol to Laboratory 2 (UCL) by the Natural History Museum, London.

Ancient Egyptian mummy tissue

The mummy of Nekht-Ankh was discovered in 1905/1906 at a burial site known as the "Tomb of Two Brothers" and belonged to Nekht-Ankh and Khnum-Nakht (David 2003). These two mummies, discovered in a previously undisturbed, provincial tomb, are archaeologically important as they have made a major contribution to the emergence and development of paleopathology, and studies on them continue. In addition to the mummies, the tomb contained a limited but well made range of artifacts. However only Nekht-Ankh was provided with a set of four canopic jars in a canopic chest, to contain his mummified viscera, removed from the body during the mummification process. The exact date of the burial cannot be determined since there is no evidence of any royal names to be found at the cemetery. The style and contents of the tomb, however, places it in the 12th Dynasty (c. 3900 BP). A small sample of liver from the canopic jar of Nekht-Ankh was examined, together with a sample of intestine and another tissue fragment from Khnum-Nakht.

DNA extraction: adult worms

A small segment was cut off and placed in 100 µl of demineralization solution (0.5 M EDTA pH 8.0/1 mg^{-1} proteinase K) in a 1.5 ml tube with 10 glass beads (1.5–2.0 mm). After vortexing, tubes were incubated overnight at 56 °C. Next the samples were incubated in lysis buffer (Qiagen DNeasy™ kit) for I hour at 70 °C. Thereafter the standard protocol was followed. DNA was captured onto spin columns, washed and eluted.

DNA extraction: ancient Egyptian mummy tissue

In Laboratory 1 DNA was extracted from the liver and intestine using a modification of the procedure described by Boom et al. (1990). Tissue was placed in 1.5 ml tubes containing 500 µl guanidium thiocyanate solution (10 M), vortex mixed for 1 min., incubated at 56 °C and gently agitated for 5 to 8 h. Next, samples were incubated at 94 °C for 10 min., centrifuged at 12000 rpm for 3 min. and the supernatant transferred to another sterile 1.5 ml tube. Guanidium thiocyanate solution (1 ml) and 10 µl of silica bead suspension were added to the sample. Tubes were vortex mixed for 20 s, placed on ice for 1 hour and agitated every 15 min. Samples were centrifuged at 12000 rpm for 30 s and the supernatant discarded. Pellets were re-suspended in 500 µl of washing buffer (2 mM Tris-HCl (pH 7.5)/10 mM EDTA (pH 8.0)/10 mM

NaCl in a 50 % (v/v) water/alcohol mixture), centrifuged for 30 s at 12000 rpm, and the supernatant discarded. This step was repeated if the silica pellet appeared discolored. The silica was washed with 200 µl absolute ethanol and air-dried. DNA was eluted with 100 µl water, mixed for 20 s, incubated at 56 °C for 1 h and re-suspended in double-distilled water. The extract was centrifuged at 12000 rpm, for 3 min. and the DNA extract stored at 4 °C. Extraction negatives were processed simultaneously to identify any contamination during this process.

In Laboratory 2 a small quantity of sample was placed in 200 µl demineralization solution in a 1.5 ml tube with beads, incubated at 56 °C and mixed in a bead beater daily until dispersed. After mixing well in a bead beater, samples were split and one 100 µl aliquot was incubated for 1 h at 60 °C with 10 µl 0.1 M *N*-phenacylthiazolium bromide (PTB), a reagent that cleaves glucose-derived protein cross-links (Poinar et al. 1998) thereby facilitating DNA release and strand separation. Both sets of samples were then incubated for 2 h at 56 °C with 200 µl of lysis buffer containing guanidium thiocyanate, EDTA and Triton X-100 (Boom et al. 1990). Subsequently the DNeasy™ protocol was followed.

PCR amplification

In Laboratory 1 the *Schistosoma mansoni* 121 bp tandem repeat sequence known as *Sm1-7* (Hamburger et al. 1991) was targeted for amplification using primers described by Hamburger et al. (1998): Primer 1: 5'-GAAAATCGTTGTATCTCCG-3' and Primer 2: 5'-GGTGACCTGCCTAAAAATAC-3'. To examine genetic relatedness between the two mummified individuals, the following primers were used: mitochondrial DNA 16210: 5'-CCC ATGCTTACAAGCAAG TA-3' (Kolman & Tuross 2000) and 16401: 5'-TGATTTCACGGA GGATGGTG-3' (Vigilant et al. 1989). Amplification was performed using a 25 µl PCR reaction mix comprised of: 20 mM Tris-HCl pH 8.4; 50 mM KCl; 2.0 mM $MgCl_2$; 2.5 mM of each nucleotide (dATP, dCTP, dGTP and dTTP); 0.25 µM of each primer; and 1.25 units of Platinum *Taq* (Gibco). DNA extract (10.0 µl) was added to each tube. PCR and extraction negative controls were included in each PCR. Amplification was achieved using a program consisting of a 3 min. initial denaturation at 94 °C, followed by 45 cycles of: 94 °C for 1 min., 55 °C (primers 1 and 2) and 60 °C (primers16210 & 16401) for 1 min., and 72 °C for 2 min.; followed by a final extension of 10 min. at 72 °C. Primers 1 & 2 produce a 46-bp product and a repetitive 121-bp product of *S. mansoni*; mitochondrial primers 16210 and 16401 amplify a 230-bp product of the hyper variable region I.

In Laboratory 2 two sets of nested pairs of primers were devised based on the *S. mansoni* tandem repeat unit (Hamburger et al. 1991) and an intergenic spacer region in S. *haematobium* near the 28S ribosomal RNA gene (Kane & Rollinson 1998):

Schistosoma mansoni outer primers SM1: 5'-GATCTGAATCCGACCAACCG-3' and SM2: 5'-CTTGTT-TTATATTAACGCCC-3' (118 bp); inner primers SM3: 5'-GAATCCGACCAACCGTTC-3' and SM4 5'-ACG-CCCACGCTCTCGCA-3' (98 bp).

Schistosoma haematobium outer primers SH1: 5'-CGCTAGACTTCGGTCTGGTT-3' and SH2: 5'-CGTCG-CAAGTATACGATAT-3' (107 bp); inner primers SH3: 5'-CTAGACTTCGGTCTGGTTAAAGC-3' and SH4: 5'-CGATATATGGATATATGTTATGC-3' (92 bp).

PCR was carried out in 50 µl volumes containing Qiagen HotStar® buffer; 10 mM bovine serum albumin; 3.5 mM $MgCl_2$; 0.5 mM of each primer; 0.2 mM of dATP, dCTP, dGTP and dTTP; and 1.25 units of HotStar® *Taq* polymerase.

Amplification was achieved using a program consisting of initial enzyme activation and DNA denaturation for 15 min. at 95 °C; followed by 10 cycles of touchdown PCR with annealing dropping 1 °C per cycle from 65 °C to 56 °C; followed by 33–43 cycles of: 94 °C for 40 s, 55 °C for 1 min., and 72 °C for 20 sec + 1 s/cycle; followed by a final extension of 1 min. at 72 °C. Nested PCR when performed used 1 µl of the stage 1 PCR product with 25 cycles of amplification.

Detection of amplified DNA

PCR product was added to loading dye and electrophoresed in a 2.0 % (w/v) NuSieve© (FMC Bioproducts) agarose gel in 1X TAE at 108 volts for 35 min. (Laboratory 1); or a 3.0 % (w/v) NuSieve© agarose gel in 1xTBE or TAE at 8.8 volts cm^{-1} for 65 min. (Laboratory 2). Amplified DNA was visualized using ethidium bromide staining exposed to ultraviolet light, and recorded with digital imaging equipment (Pharmacia Biotech, ImageMaster or LabWorks 3.6). PCR products were excised from the agarose using sterile implements for direct sequencing.

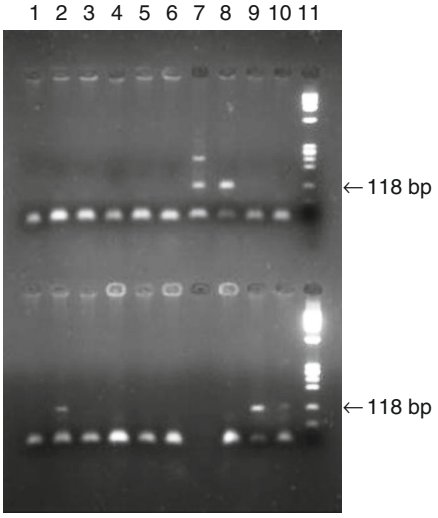

Fig. 2. Gel electrophoresis of *Schistosoma mansoni* PCR. Wells were loaded as follows: Top row were DNA extractions with phenacylthiazolium bromide (PTB); bottom row were extracted without PTB. Lane 1; unrelated archaeological sample; lane 2: Nekht-Ankh liver; lane 3: Khnum-Nakht intestine; lanes 4–6: unrelated archaeological samples; lane 7 (top row): gel positive control – archival stored PCR product; lane 7 (bottom row): blank; lane 8 (top row): DNA extraction positive control – archived; lane 8 (bottom row): DNA extraction negative control; lane 9 (top row): DNA negative control; lane 9 (bottom row): DNA extraction positive control; lane 10 (top row): PCR negative control; lane 10 (bottom row): PCR positive control – archived DNA extract; lane 11: molecular markers – PhiX174*HaeIII* digest. The 118 bp *S. mansoni* PCR product can be seen in lanes 7 and 8 (top row) and lanes 2, 9 and doubtfully 10 (bottom row). Negative and positive controls are satisfactory and the extract from the Nekht-Ankh liver is weakly positive.

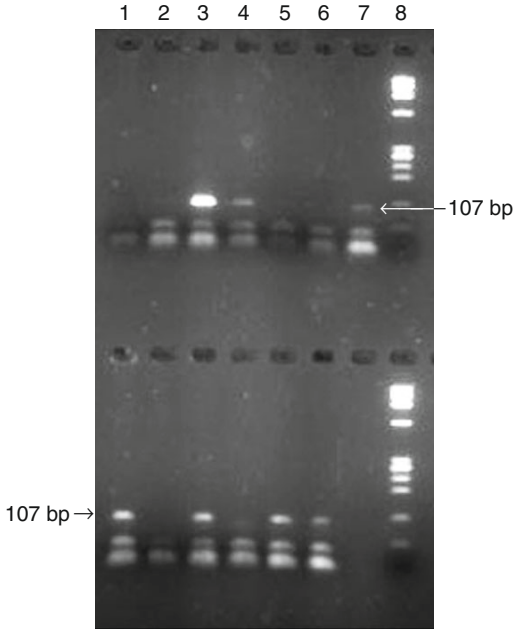

Fig. 3. Gel electrophoresis of *Schistosoma haematobium* PCR. Wells were loaded as follows: Top row: lane 1: PCR negative control; lane 2: DNA extraction negative control; lane 3: *S. haematobium* positive control – 1 : 100 000 dilution of worm extract; lanes 4–6: unrelated archaeological samples; lane 7: Nekht-Ankh liver. Bottom row: lanes 1–4: samples from four unrelated canopic jars; lanes 5–6: Khnum-Nakht intestinal samples; lane 7: blank. Lane 8 (both rows): molecular markers – PhiX174*HaeIII* digest. Negative and positive controls are satisfactory and the extracts from the Nekht-Ankh liver, the Khnum-Nakht intestinal samples, two of the canopic jar samples and another unrelated archaeological sample are weakly positive.

DNA sequencing

In Laboratory 1 the PCR product was directly sequenced using a radioactive manual sequencing kit (Sequenase Kit, Amersham). Sequencing PCRs consisted of ^{32}P radio-labeled nucleotides, Sequenase buffer and 4 µl of liquefied (94 °C) excised agarose PCR product to a total reaction volume of 10 µl. Amersham recommended thermocycling parameters were used and radio-labeled DNA was run on a 6 % acrylamide gel and exposed to high resolution X-ray film (Kodak) for 48 to 72 hours before development.

Results

Optimization and verification experiments

The *S. mansoni* PCR primers were highly specific and no amplicons were detected with *S. haematobium* or *S. intercalatum* DNA extracts. The *S. haematobium* primers generated some amplified DNA from the *S. intercalatum* DNA extract. This could be reduced considerably by touchdown PCR.

Ancient Egyptian mummy tissue

In Laboratory 1 extraction of DNA from the liver and intestine samples was immediately followed by PCR amplification of the 121-bp *S. mansoni* tandem repeat unit. A faint 121-bp product was amplified from the Egyptian liver sample but subsequent attempts to repeat amplification were unsuccessful. It was noted that significant degradation of the DNA extract occurred during a 5-hour period before a second amplification was attempted. Due to sample limitations this extraction could not be replicated. A sequence exhibiting 100 % identity with *S. mansoni* (reference sequence NCBI nucleotide accession #: M61098) was obtained. The HVII mitochondrial DNA was successfully amplified from both individuals (Table 1). All extraction and amplification controls were negative.

Table 1. Hypervariable region I mitochondrial polymorphisms.

Individual	16223 C→T	16261 C→T	16292 C→T
Nekht-Ankh	+	+	+
Khnum-Nakht	+	–	+

In Laboratory 2 a faint 118-bp *S. mansoni* product was obtained from the Nekht-Ankh liver sample, using the DNA extracted without the facilitating reagent PTB. No amplicons were obtained with the Khnum-Nakht intestine samples. All extraction and amplification controls were negative (Fig. 2). However, *S. haematobium* DNA was detected in both the Nekht-Ankh liver and two samples from Khnum-Nakht, believed to be intestinal (Fig. 3). There was insufficient material for further DNA extractions.

Discussion

Results confirmed that the DNA extraction procedures resulted in amplifiable DNA from fixed tissues, without the need for PTB. The sensitivity of PCR amplification was demonstrated by Pontes et al. (2002) who, using a similar nested PCR and purified DNA, showed that as little as 1 fg was detectable. The outer and nested *S. mansoni* primers were specific. However, the *S. haematobium* PCR allowed some *S. intercalatum* amplicon unless stringent conditions were employed. This appears to be a general problem, as Hamburger et al. (2001) reported a similar finding based on the *Dra1* repeat sequence.

S. mansoni DNA was amplified and sequenced from the liver of a 3900 year old Egyptian mummy, Nekht-Ankh, suggesting that he suffered from chronic schistosomiasis. This was confirmed in two independent laboratories. The intestinal samples from Khnum-Nakht were positive for *S. haematobium* but were negative for *S. mansoni*. These findings support previous microscopic observations of *Schistosoma* spp. eggs and immunocytochemistry (Rutherford 1999, David 2003). Data from Laboratory 2 indicate that the liver sample from Nekht-Ankh was also positive for *S. haematobium*. In addition, *S. haematobium* DNA was present in material from two of four unrelated Middle Kingdom canopic jars, which confirms earlier conclusions that this species was widespread in ancient Egypt. Although *S. haematobium* eggs are usually found in the ureter and bladder, the early observations by Ruffer (1910) described the calcified ova of *Bilharzia haematobia* in the straight tubules of the kidney, whilst Reyman et al. (1977) reported schistosome eggs in the kidneys of a teenage boy, Nakht. Both these reports also described cirrhosis of the liver, and calcified schistosome eggs with terminal spines, typical of *S. haematobium*, were seen in histological sections of tissue from the portal region of the Nakht liver (Nunn & Tapp 2000). Therefore, although the presence of this species is less usual in the liver, our finding is in line with the earlier reports. In addition, where there are high intensities of *S. haematobium* and mixed infections with *S. mansoni*, *S. haematobium* can be found in the mesenteric veins and thus their eggs appear in the liver and gastrointestinal tract (Cunin et al. 2003).

It is believed that schistosomiasis originated in Central Africa and that the disease spread from Ethiopia down the Nile (Adamson 1976). The snail hosts of *S. haematobium*, *Bulinus* spp, are bottom feeders and are thus relatively unaffected by the rate of flow of the river. However, the snail hosts of *S. mansoni*, *Biomphalaria* spp, are surface feeders, and cannot establish themselves except in waters with a slow current or none. Therefore, it was believed that *S. haematobium* was always prevalent in Upper Egypt, whereas *S. mansoni* was concentrated in the Nile delta (El-Khoby et al. 2000). Recently Hibbs et al. (2011) demonstrated *S. mansoni* in desiccated tissue from Wadi Halfa (350–550 CE) and Kulubnarti (550-750 CE) – settlements in early Christian Nubia, using a specific enzyme-linked immunosorbent assay that does not cross-react with *S. haematobium*. There was a clear association between incidence of

Fig. 4. Margaret Murray and her team unwrap the mummy of Khnum-Nakht in 1908. On May 6 1908 Dr. Murray and her scientific team carried out an autopsy on the mummy of Khnum-Nakht in the Chemistry Lecture Theatre, University of Manchester. There was an invited audience of 500 people from the Manchester Egyptian Association (David 2003). This was a critical event in the development of paleopathology as a scientific discipline.

S. mansoni and local irrigation practices in Wadi Halfa and the authors pointed out the need for further data on the distribution of *S. haematobium* in the Nile Valley. The present study confirms the prevalence of *S. haematobium* and also provides the first direct evidence of *S. mansoni* from 3900 years ago. The tomb of Nekht-Ankh and Khnum-Nakht was in Rifeh, near Assiut in Central Egypt. This may have been an area where both species of *Schistosoma* were prevalent, as there almost certainly would have been pools, wells and irrigation channels in the area that could have provided a source of *S. mansoni* (Butzer 1976).

The mitochondrial DNA sequences obtained from Nekht-Ankh liver and Khnum-Nakht intestinal samples (Table 1) exhibit the 16223 T and 16292 T mutations common in the Middle East and Egypt. Nekht-Ankh also exhibited the 16261 T mutation, suggesting that these two "brothers" were not maternally related. Exploration of postmortem damage of endogenous DNA has characterized genetic damage, including oxidative modification and hydrolytic deamination, in ancient human samples as 'miscoding lesions' (Gilbert et al. 2003). Although results from the HVI mitochondrial analysis have not been independently verified, they were replicated and showed no evidence of polymorphic variation, in either the electropherograms or autoradiographs. In addition, the preservation of proteins and lipids in a skin sample from Nekht-Ankh has been shown to be good (Petersen et al. 2003), which suggests that the positive DNA results are likely to be genuine. Therefore it was concluded that authentic polymorphisms were identified from these Egyptian mummies. The difference in the strength of the reactions from the schistosomal DNA may reflect the limited and localized distribution of the parasite compared with the human tissue.

In the inscriptions on the tomb equipment both "brothers" were described as the sons of a local governor or mayor, and this wealthy family would have enjoyed considerable local status. It is recorded that they were the sons of a woman named Khnum-Aa who carried the title "mistress of the house". However, the inscriptional evidence may indicate that Nekht-Ankh and Khnum-Nakht were half broth-

ers, the children of different fathers since in Khnum-Nakht's case it provides information that both his father and paternal grandfather were local governors, whereas it only states that Nekht-Ankh's father held that position. The inscriptions also record that Khnum-Nakht was a "great *wa'ab* priest" who served the ram-headed god Khnum, and it would have been customary for Khnum-Nakht, as the son of the local governor, to become a priest in the local temple. There is no information regarding the career of Nekht-Ankh.

The original study in 1908 considered the vexed question of the parentage of the "brothers" as there are marked differences between the brothers' skeletons particularly their skulls – Khnum-Nakht's skull was prognathous, while that of his brother was orthognathous. Adoption was not uncommon in ancient Egypt and this was thought to explain their physical dissimilarity. The findings of the present study that these two individuals are maternally unrelated are consistent with these morphological observations.

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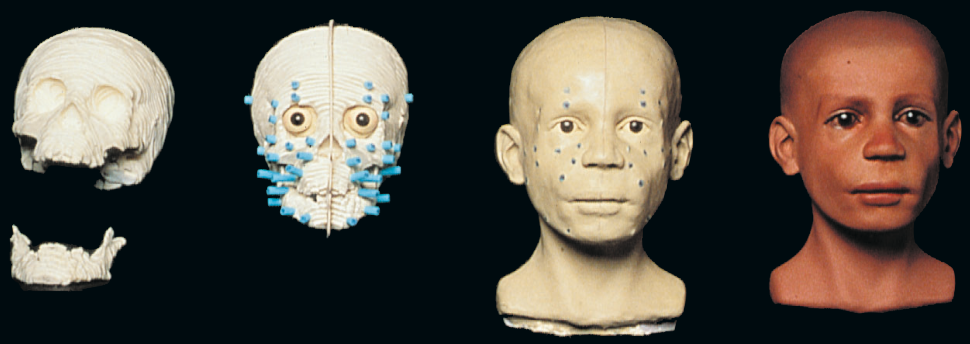

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CT-based 3D reconstruction of the Late Period Egyptian mummy of Tadimentet inside the coffin.
(Daizo et al., pp. 71–76)

Back cover

Stages of the facial reconstruction of a Roman Period Egyptian child mummy
made by Raymond Evenhouse (University of Illinois at Chicago) in 1990.
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