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Abstract. The teeth and skulls of two ancient Egyptian mummies, the 'Two Brothers' located in the Manchester Museum have recently been re-examined. Their bodies were originally discovered buried alongside each other in a tomb at Deir Rifeh in Egypt, and these two individuals were considered to be related. Similar to most ancient Egyptians the teeth display considerable tooth wear but lit-

tle evidence of carious lesions. Khnum-nakht, the younger of the two, presents the rare developmental disorder of fusion of the left maxillary incisor and gemination of the right incisor, probably the earliest recorded instance of this irregularity. Analysis of the DNA from their molar teeth has been able to shed some light on the longstanding question of the kinship of these two mummies.

Keywords. Mummies. Ancient Egypt. Kinship. Teeth. DNA.

#### I. INTRODUCTION

The 'Two Brothers', two ancient Egyptian mummies, are among the more well-known inhabitants of the Manchester Museum, where they have been on almost continuous display since they were first acquired by the museum over a hundred

years ago. Early in 2018 they were the centre of much media interest both in the United Kingdom and further afield, for the results of their DNA analyses which has been able to shed some light on their kinship, a question that has puzzled and intrigued Egyptologists ever since their discovery.

The 'Two Brothers' were known as such, as hieroglyphic inscriptions on the wooden sarcophagi of the two men, indicate that they were born of the same mother, Khnum-Aa, and their unnamed father was a *hatia* prince or nomarch (Fig. I). The bodies were found side by side in a tomb that has been dated to the middle of the Twelfth Dynasty (c. 1900-1800 BC). The intact tomb chamber was discovered in 1907 at Deir Rifeh, a village some 250 miles south of Cairo.

The burial was unearthed by an Egyptian workman under the supervision of British Egyptologists, Flinders Petrie and Ernest Mackay. The complete contents of the find, including their coffins and grave goods, were later transferred to Manchester Museum in 1908, and remained intact as a group rather than being divided among different museum collections, as was usual at that time (David 1979: I and 2007).



**Fig. 1.** The 'Two Brothers' inner 'body' coffins – Khnum-nakht on left and Nakht-ankh on the right. (Courtesy of Manchester Museum, the University of Manchester).

### 2. EARLY INVESTIGATIONS

Upon arrival in Manchester in 1908, the mummies of the 'Two Brothers', Khnum-nakht and Nakht-ankh were unwrapped by the UK's first female Egyptologist, Margaret Murray who was also the first curator of the Egyptian collection at the Manchester Museum. In an important development in scientific study, Margaret Murray assembled a multi-disciplinary team of researchers to examine their bodies in what was one of the earliest full-scale scientific investigations carried out on mummies (Murray 1910).

Dr John Cameron, a medical member of the team, undertook the post-mortem examination of the mummified bodies and he concluded that Nakht-ankh was at least 60 when he died, and with the average life expectancy in ancient Egypt being around thirty-five, Nakht-ankh had experienced a relatively long life. Khnum-nakht on the other hand probably died in his forties. However, the assessment of age was based solely on the degree of fusion of the sagittal and coronal sutures. Although cranial sutures generally fuse with increasing age, there is considerable variability in closure rates and patterns, and so care has to be taken in basing conclusions solely on these estimations. Today it is recognised that the determination of age by traditional morphological methods is often imprecise (Mays 2010: 40-90; Villa & Lynnerup 2014: 3-9).

Stature for both of the mummies was estimate at about 1.60 metres and they both displayed evidence of osteoarthritis in the vertebrae. The teeth were briefly commented on, particularly the fused left maxillary incisors of Khnum-nakht which was likened at the time to a 'huge tusk' (Cameron 1910).

#### 3. CRANIAL EVIDENCE

An examination of the skulls by Dr Cameron revealed that they were quite different with that of Khnum-nakht being noticeably prognathous and Nakht-ankh orthognathous. The protrusive nature of both the maxilla and mandible of Khnum-nakht resulted in the conclusion that he was of black African descent, possibly Nubian. This divergence in skeletal morphology was found to apply to many of the other anatomical features evident in the skulls (Fig. 2). The shape and size of the calvarium, and the dimension of the zygomatic bones, orbits and nasal cavities were all recorded as displaying a marked metric variation. Similarly, this also extended to the postcranial skeleton and led to the conclusion at the time that it was unlikely





Fig. 2. Skulls of the 'Two Brothers', Nakht-ankh on left and Khnum-nakht skull on the right. (Courtesy of Manchester Museum, the University of Manchester).

that they were directly related. Dr. Cameron commented that: 'these differences are so pronounced that it is almost impossible to convince oneself that they belong to the same race, far less to the same family'. In the 1970's radiological examinations of the brothers, unavailable at the time of the original investigation, were undertaken together with facial reconstructions, which again highlighted the variation in cranial morphology.

Inheritance is an important factor in craniofacial morphology and familial studies have demonstrated that craniofacial shape shows a moderate to high degree of heritability for a substantial set of traits including facial height and width; nasal and orbital features, and tooth morphology (Naini & Moss 2004; Johannsdottir et al. 2005; Martínez-Abadías et al. 2009).

Cranial morphology is a complex interaction of intrinsic (such as hormonal and genetic) and extrinsic (mainly environmental and mechanical) factors. Taking into consideration the dental developmental anomalies (described below), it is quite possible that the cranial development of one or both individuals may have been perturbed, leading to pronounced differences in adult cranial form. Therefore, the influence of inheritance on craniofacial traits cannot, by itself, be used to assess a familial relationship, as was suggested at the time of the 1908 investigation.

### 4. TEETH

Late in 2017 the teeth of the 'Two Brothers' were re-examined by the author as part of a new investigation into the relationship of these two individuals. An examination of ancient teeth is particularly useful in archaeological studies as it can provide considerable information related to the life style and dietary habits of ancient peoples. Visual and radiological studies can identify palaeopathological lesions useful in identifying dietary patterns. Plant microfossils and non-dietary debris are able to be isolated from calculus and can be identified using light microscopy, again providing dietary evidence as well as cultural and environmental data. More recent analytical techniques such as stable isotope analysis can assist in tracing the geographic origins and migrations of peoples. In addition, there are a number of anthropological questions that can potentially be addressed by DNA analysis, such as the determination of sex when conventional identification methods are impossible, as well as the origin, familial relationships and movements of populations (Forshaw 2014).

The visual examination of the dentitions of the two mummies indicated that Khnum-nakht would have possessed a full complement of teeth during life whereas Nakht-ankh lost his maxillary right lateral incisor ante-mortem. The extensive loss of labial and buccal bone around the socket of this missing lateral incisor and its adjacent teeth, suggest that its premature loss may be due to trauma, perhaps an accident or interpersonal violence. As is the case with many ancient skulls, numbers of their teeth are now missing, presumably lost in post-mortem handling.

### 4.1. Caries

Similar to many dentitions from ancient Egypt and other ancient societies there is very little evidence of caries in the teeth of the 'Two Brothers', with only one small cavity present in the occlusal surface of the right mandibular third molar of Nakht-ankh. This low incidence of caries is primarily due to a lack of fermentable carbohydrates in the ancient diet and to a lesser extent the excessive tooth wear. Such wear on the occlusal surface of the teeth would eliminate the natural pits and fissures, stagnation areas that are prone to carious attack. Similarly, interproximal wear would have produced flattened tooth contacts, creating a more difficult environment for plaque and caries to proliferate in (Forshaw 2009: 423). Another factor was the fibrous abrasive nature of the food, which would tend to inhibit the retention of plaque on the tooth surfaces (Marion 1996: 15).



Fig. 3. Teeth of Khnum-nakht showing extensive tooth wear.

### 4.2 .Tooth wear

The teeth of both Nakht-ankh and Khnum-nakht display heavy occlusal tooth wear, again a common finding in the majority of ancient Egyptian dentitions (Ruffer 1920: 348; Leek 1967: 51; Hillson 1979: 156; Forshaw 2009: 421). This wear has resulted in complete loss of enamel and significant loss of dentine from the occlusal surfaces of all of the teeth (Fig. 3). The primary cause of this tooth wear was the chewing throughout life of a coarse fibrous diet made even more abrasive by the introduction of inorganic particulate matter, particularly into the bread, the staple food of the ancient Egyptians. These particles were present largely as a result of contamination of the grain by wind-blown sand and also by other factors such as the use of flint-tooth sickle harvesting tools and grinding grain with soft sandstone implements (Forshaw 2010: 72).



Fig. 4. Maxillary anterior teeth of Khnum-nakht. The central incisors display the rare developmental abnormality of fusion (left) and gemination (right) within the same arch.

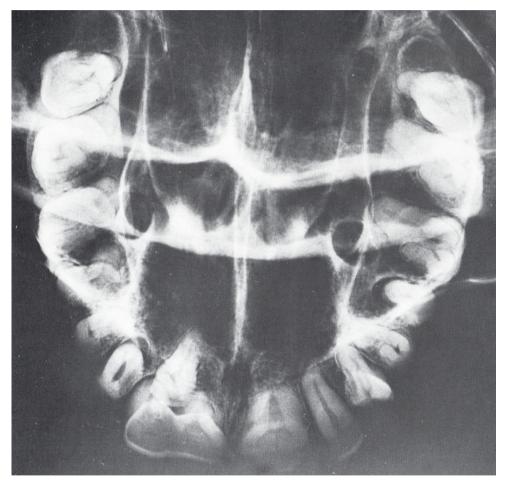
### 4.3. Developmental anomalies

The maxillary anterior teeth of Nakht-ankh present a normal appearance but both the maxillary central incisors of Khnum-nakht are abnormally large with the left incisor displaying a vertical groove, extending from the incisal edge to the root apex (Fig. 4). These central incisors demonstrate the rare developmental condition of fusion of the left incisor and gemination of the right incisor, and this instance of gemination and fusion within the same individual and dating back nearly four thousand years is the earliest known recorded example of such an anomaly.

Gemination and fusion are developmental anomalies of tooth form, which affect both the permanent and primary dentitions (Tannenbaum & Alling 1963; Duncan & Helpin 1987; Nandini et al. 2014; Slootweg 2016: 179-80; Berkovitz et al. 2017: 364). Gemination is recognised as an unsuccessful attempt by a single tooth germ to divide by invagination during the proliferative stage of dental development. This results in a large single tooth whose appearance varies from a groove or depression to two crowns joined together, indicating the abortive attempt of the teeth to be completely separate. Geminated teeth have a single

pulp chamber and usually a large single root and root canal, as is the case with Khnum-nakht (Fig. 5).

Fusion is a union of two separate tooth germs at some stage in their development and is suggested to be the result of some physical force or pressure. The fusion may be partial or total depending upon the stage of tooth development at the time of union, and so one tooth may be present with only one pulp chamber



**Fig. 5.** Occlusal radiograph of the maxillary teeth of Khnum-nakht. The left central incisor is fused with a supernumary tooth and the lateral incisor is instanding due to lack of space within the dental arch. The right central incisor is geminated. (Courtesy of Manchester Museum, the University of Manchester).

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and a merging of dentine and/or enamel, as in gemination, or there may be two separate pulp chambers with two roots or two canals in a single root. In the case of Khnum-nakht the left central incisor shows separate pulp chambers and separate roots (Fig. 5).

Fusion can occur between teeth of the same dentition or mixed dentitions, and between normal and supernumerary teeth (Peyrano & Zmener 1995: 196; Nunes et al. 2002: 140). Supernumerary teeth are often atypical and so fusion between a supernumerary and a normal tooth will generally show differences in the two halves of the joined crown, whereas in examples of gemination the two halves of the joined crown are commonly mirror images, as with Khnum-nakht. Despite the considerable number of cases reported in the literature, the differential diagnosis between these abnormalities can on occasions be difficult, particularly when supernumerary teeth are present, and so diagnosis requires careful visual and radiographic examination (Jain et al. 2014; Carmago et al. 2016).

When fusion occurs, the total number of teeth in the dental arch will be reduced unless a supernumerary tooth is involved. With Khnum-nakht the maxillary left central incisor has fused with a supernumerary tooth, and the left lateral incisor is palatally displaced due to lack of space within the dental arch, resulting in no reduction in the number of teeth. Gemination by definition will not reduce the number of teeth present.

The exact causes of these abnormalities are uncertain but gemination is believed to be the result of a disturbance in epithelial-mesenchymal interactions which can markedly disturb tooth development (Berkovitz et al. 2017: 364). It is suggested that this may be caused by local metabolic interferences occurring during morpho-differentiation of the tooth germ. Severity of the anomaly depends on the stage of formation of the involved teeth (Grover & Lorton 1985: 313). With fusion the influence of pressure or physical forces producing close contact between two tooth germs during their development can be a factor (Jain et al. 2014; Carmago et al. 2016: 73). As the aetiology remains unclear, to avoid any confusion, some authors prefer to use the term 'double teeth' which describes the appearance with no implication regarding the cause of this condition (Killian & Kroll 1990: 575; Olivan-Rosas et al. 2004: 226).

These dental abnormalities are more frequent in the anterior region of the permanent dentition, they do not show a sex predilection but genetic predisposition and racial differences have been cited as contributing factors in both gemination and fusion (Duncan & Helpin 1987: 85-87; Jain et al. 2014). While the

incidence varies in individual reports, for single examples it appears to be approximately 0.5% in the primary dentition and 0.1% in the permanent dentition. Bilateral presentation of these conditions is even less common with prevalence estimated at 0.02% - 0.05% for both dentitions (Duncan & Helpin 1987; Aguiló et al. 1999; Türkaslan et al. 2007: 188). In both primary and permanent dentitions fused or geminated teeth may cause functional, aesthetic, caries, periodontal and orthodontic problems. Apart from the 'Two Brothers' there appears to be no other documented instance of this double developmental abnormality in ancient specimens.

### 5. DNA ANALYSIS

Teeth are an excellent source of DNA as being located within the mandible and maxilla they are largely shielded from the environmental and physical conditions that act to accelerate the process of post-mortem decomposition and DNA decay (Schwartz & Schwartz 1991; Alvarez Garcia et al. 1996: 125). DNA extracted from teeth and particular the cementum of teeth, where concentrations are higher, is less prone to contamination than DNA extracted from bones.

In 2018 two molar teeth were removed from both Nakht-ankh and Khnumnakht, and under carefully controlled laboratory conditions, cementum and dentine was obtained from the roots of these teeth by means of the 'reverse root canal technique' (Cobb 2002; Alakoç & Aka 2009). The minimally destructive nature of this procedure preserves the crown of the tooth intact.

DNA was then extracted from the tooth powder by utilising standard methodology by Dr. Konstantina Drousou in the Manchester Institute of Biotechnology (Drousou et al. 2018). Using Second Generation Sequencing she was able to determine a maternal relatedness between the two individuals consistent with a shared mother or a more distant kinship relationship such as cousins or unclenephew.

### 6. CONCLUSION

The results of the examination of the teeth of two ancient Egyptian mummies revealed not only extensive tooth wear and a lack of carious lesions, common findings in ancient Egyptian teeth, but also the extremely rare double developmental disorder of gemination and fusion, perhaps the earliest recorded example of such an abnormality. In addition, ancient DNA was successfully extracted from

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their molar teeth which indicated a maternal relatedness. The DNA results thus add weight to the ancient inscriptional evidence supporting a familial relationship between the 'Two Brothers'. Consequently, the teeth of the 'Two Brothers' has been able to furnish further information on the kinship of these two ancient Egyptians who lived some four thousand years ago in the Nile valley, a quandary that has perplexed Egyptologists for over a hundred years.

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