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Abstract: Previous analysis of the perinatal infant burials from Romano-British Hambleden indicated the practice of infanticide at that site. We attempted to determine whether this practice was specifically targetted at one sex or other by determining the sex of the infants using analysis of fragments of the amelogenin gene. We also analysed mtDNA in order to shed light on aspects of kinship. Thirty-three infants were analysed, and sex was successfully identified in 12. Seven were female, five male. No two infants shared identical mtDNA polymorphisms, indicating that all came from different mothers. Taken together with previous DNA results from perinatal remains from Roman-British sites where infanticide has been identified, they provide no evidence that manipulation of the sex ratio was a motivation for infanticide in Roman Britain.

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Covering Letter

I hearby submit the paper entitled 'Ancient DNA study of the remains of putative infanticide victims from the Yewden Roman villa site at Hambleden, England' for consideration for publication in Journal of Archaeological Science

***Highlights (for review)**

DNA analysis of 33 infant burials from Roman Hambleden, England, where previous work had identified infanticide

Sex was identified in 12; seven were female, five male

None shared mtDNA haplotypes in common

The current results provide no evidence that infanticide was used to manipulate sex ratios in Roman Britain

*Manuscript
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Ancient DNA study of the remains of putative infanticide victims from the Yewden Roman villa site at Hambleden, England

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ABSTRACT

Previous analysis of the perinatal infant burials from Romano-British Hambleden indicated the practice of infanticide at that site. We attempted to determine whether this practice was specifically targetted at one sex or other by determining the sex of the infants using analysis of fragments of the amelogenin gene. We also analysed mtDNA in order to shed light on aspects of kinship. Thirty-three infants were analysed, and sex was successfully identified in 12. Seven were female, five male. No two infants shared identical mtDNA polymorphisms, indicating that all came from different mothers. Taken together with previous DNA results from perinatal remains from Roman-British sites where infanticide has been identified, they provide no evidence that manipulation of the sex ratio was a motivation for infanticide in Roman Britain.

Keywords: aDNA; sex determination; amelogenin; mtDNA; kinship

1. Introduction

Infanticide is the killing of unwanted infants. Until recently, it was a practice that was tolerated in most human societies around the world (Divale and Harris, 1976). Prior to the advent of effective contraception, it was one of the few ways of limiting family size that was both effective and safe for the mother. Infant life is readily extinguished by methods such as smothering which involve little overt violence, so infanticide usually leaves no traces upon the bones. Indirect methods are required for its identification in the archaeological record. Because it is usually carried out immediately after birth, the regular practice of infanticide would be expected to lead to a perinatal age at death distribution which resembles the gestational age of total live births – i.e. tightly clustered around an age approximating to a full-term baby. The age distribution of natural deaths in the perinatal period (stillbirths, natural deaths in the weeks immediately following birth) tends to be more dispersed, with less of a peak at full-term. Age at death in the perinatal period can be accurately estimated from long-bone lengths, so this demographic method may be used to identify infanticide from skeletal remains (Mays, in press).

Written sources indicate that infanticide was practiced in classical Roman society (Harris, 1982; Wiedermann, 1989). Palaeodemographic evidence, of the type described above, has been used to argue for its occurrence in Britain when it was a province of the Roman Empire (Mays, 1993, 2003; Mays and Eyers, 2011; Bonsall, in press), and formed part of the evidence attesting to infanticide at the site of a brothel at a bathhouse in Roman Ashkelon, Israel (Smith and Kahila, 1992).

Infanticide allows sex-selection of which offspring are to be raised, and in societies where children of one sex are valued more than the other, the regular practice of infanticide may lead to skewed sex ratios. In Roman society, there is evidence from written sources that more girls than boys may have been victims of infanticide (Lewis, 1985: 54; Wiedermann, 1989: 37; Watts, 2001). It has long been noted that males outnumber

females among adults in Romano-British cemeteries. Female infanticide might be an explanation, but it is only one of various possibilities (Mays, 1995; Davison, 2001; Watts, 2001). In order to investigate more closely the existence of sex-selective infanticide, sex identification of infants from burial sites where there is independent evidence for infanticide is needed.

Sex identification from bony morphology is problematic in non-adult individuals (Mays and Cox, 2000; Lewis, 2007: 50-54; Saunders, 2008). However, the value of ancient DNA (aDNA) in this regard is well established, the usual method being analysis of the amelogenin gene as the X and Y chromosomes carry different alleles (Brown and Brown, 2011: 157-167). Previous application of this method to skeletal remains of putative infanticide victims from Roman Britain (Mays and Faerman, 2001) failed to produce evidence of a sex imbalance. The current work is an aDNA study of likely infanticide victims from the Yewden Roman villa site at Hambleden, England. The aim of the work is to contribute to the study of sex-selective infanticide in Roman Britain, and also to attempt to evaluate reasons for infanticide at that particular site.

Infant burials are frequently found on Romano-British domestic sites but the Yewden villa site, Hambleden, has yielded the largest number from a habitation site from the Roman period in Britain. The excavator (Cocks, 1921) noted 97 infant burials, and the sheer numbers led him to suggest infanticide as a possible explanation. Subsequent commentators (e.g. Watts, 1989; Philpott, 1991: 98; de la Bédoyère, 1993: 84) have repeated this interpretation. It has also been postulated that the infants were the unwanted female offspring of slaves at the site (Frere, 1967: 266-7) despite there being no evidence for slavery present at this site or any nearby centres. The interpretation of infanticide recently received support from a demographic study of the Hambleden infant remains. The distribution of perinatal ages at death was compared to that at another site (Roman Ashkelon) where earlier work (Smith and Kahila, 1992) showed evidence for infanticide,

and Mediaeval Wharram Percy, England, where previous studies (Mays, 1993, 2002; Lewis and Gowland, 2007) demonstrated a dispersed gestational age distribution consistent with natural deaths. Comparisons with these sites were carried out using four different methods: direct comparison of the distribution of long-bone lengths, and comparison of ages at death estimated using two different regression techniques and using a Bayesian methodology. Results consistently showed strong clustering around an age corresponding approximately to a full term child. This resembled the distribution observed in the Ashkelon case, supporting the case for infanticide at Hambleden (Mays and Eyers, 2011).

The Hambleden results resemble those from other Romano-British sites (Mays, 1993; Bonsall, in press), and it may be that this is simply another manifestation of the widespread, regular practice of infanticide in Romano-British society. However, an alternative explanation, that Hambleden was the site of a brothel, and that the perinatal infants were the result of infanticide of unwanted babies among its employees, has also been suggested (Eyers, 2011: 278). Brothels in the Roman Empire were normally in towns, but the Hambleden villa occupied an important river-crossing point, so passing trade may potentially have made such a business viable. As noted above, infanticide was identified at the Roman Ashkelon brothel site where remains of nearly 100 perinatal infants were found (Smith and Kahila, 1992). There, aDNA analyses identified an excess of male infants, the suggestion being that prostitutes selectively reared girls to join the trade, with the mostly male remainder being discarded (Faerman et al., 1998). A finding of an excess of males among the Hambleden infant burials would be contrary to a general preference for male offspring in the Roman World, but would potentially be consistent with the brothel theory.

In addition to identifying sex using the amelogenin gene, we also investigate kinship among the infants using mitochondrial DNA (mtDNA) sequencing. In the absence of

effective contraception, employees at a brothel would have been likely to incur repeated pregnancies. Because mtDNA is inherited maternally, it may be hypothesised that this would mean that infants were more likely to have mtDNA haplotypes in common. If, on the other hand, the infant burials were the product of infanticide by different mothers who were not necessarily maternally related, then one might expect greater heterogeneity in infant mtDNA haplotypes.

2. Materials and methods

2.1 Bone samples

Yewden Roman villa dates from the 1st-4th century AD. The 97 infant burials noted in the original site report (Cocks, 1921) mainly come from an open 'yard' area situated immediately to the north of the buildings of the villa complex. Despite the reported 97 infant burials, the site archive contains only 35. The full reasons for the discrepancy are uncertain, however one factor seems to be that Cocks included, in his total, finds of isolated bones or bone fragments which in all probability represent redeposited material rather than remains of *in situ* inhumation burials. Loss of material is also a possibility during the 1912 collection, during subsequent processing and packing, and in several movements of the collection between buildings during their storage from 1912 to the 1960s, with the final transfer to the Buckinghamshire County Museum where they have only recently been catalogued formally.

Bone samples were taken from 33 of these infants (Supplementary Table 1) with a hacksaw or electronic drill by personnel wearing protective clothing including forensic suits, hair nets, face masks and two pairs of sterile gloves. Samples were placed in sterile plastic bags and stored under dry and cool conditions.

2.2 Ancient DNA authentication regime

Ancient DNA analyses were performed in the aDNA laboratories of the University of Manchester and Complutense University of Madrid, Spain. Work in Manchester was carried out in a suite of independent, physically isolated laboratories, each with an ultrafiltered air supply maintaining positive displacement pressure and a managed access system. All surfaces within the laboratories were periodically sterilized by UV irradiation and cleaned with 5% bleach and 70% ethanol, and all utensils and equipment were treated with DNA-Away (Molecular Bioproducts) before and after use. Items such as test tubes were UV irradiated (254 nm, 120,000 µJ cm^{-2} for 2 × 5 min, with 180° rotation between the two exposures) before use. Aqueous solutions were similarly irradiated for 15 minutes. Personnel wore protective clothing including forensic suits, face masks, hair nets, goggles and two pairs of sterile gloves at all times. DNA extractions were carried out in a Class II biological safety cabinet in one laboratory within the facility, and PCRs were set up in a laminar flow cabinet in a second, physically-isolated laboratory. Work in Madrid was carried out in physically separated laboratories for DNA extraction and PCR set-up, both UV irradiated before and after each work period. Surfaces and laboratory equipment were regularly cleaned with bleach. Personnel wore disposable laboratory coveralls, masks, caps, glasses, shoe covers and gloves. All reagents and consumables were DNase and RNase free. All procedures were carried out in a laminar flow cabinet previously cleaned with bleach and UV irradiated. All DNA extractions were accompanied by three blanks (normal extraction but without skeletal material) per seven samples (Manchester) or one blank per seven samples (Madrid). Every set of 5–7 PCRs was accompanied by at least two blanks (set up with water rather than DNA extract).

2.3 DNA analysis

To remove external contamination from bone samples (Bouwman et al. 2006), approximately 1–2 mm of the outer surface was removed mechanically, and the remaining

sample UV irradiated (254 nm, 120,000 µJ cm^{-2}) for 2 × 10 min, with 180° rotation between the two exposures, followed by placing in 4% bleach solution for 2 min and washing in Millipore water. Each sample was left to dry, and then placed in a DNA-free plastic bag wrapped in a sterile piece of aluminium foil and crushed into fine powder. At least two independent DNA extractions were performed, each with 0.5 g bone powder, as described by Bouwman and Brown (2005).

Duplicate PCRs were carried out with the mtA, mtC, mtD, mtF, mtG, mtV or mtW primer pairs, which together amplify positions 15,996–16,420, 16–132 and 232–368 of the mtDNA hypervariable I and II regions (Bouwman et al., 2008), using the Multiplex PCR kit (Qiagen) in 25 µl volumes containing 5 µl DNA extract and 100 ng each primer. Cycling conditions were: 15 min at 95°C; followed by 44 cycles of 90 sec at x°C, 90 sec at 72°C and 30 sec at 94°C; followed by 90 sec at x°C and 15 min at 72°C, where x°C is the primer-specific annealing temperature (Bouwman et al., 2008). PCR products were examined by electrophoresis in 2% agarose gels. The positive samples were purified using Qiaquick columns (Qiagen), and DNA was ligated into pCR4 TOPO TA (Invitrogen) and cloned using the StrataClone PCR cloning kit (Stratagene). DNA from recombinant colonies was reamplified, purified using Qiaquick columns and sequenced with the ABI Big Dye sequencing kit (Applied Biosystems). DNA sequences were aligned with the revised Cambridge Reference Sequence (rCRS) (Andrews et al., 1999) using BioEdit (Hall, 1999), and haplogroups were assigned following established rules (http://haplogrep.uibk.ac.at/).

PCRs were also directed at the amelogenin gene, for sex identification, and eight autosomal miniSTR markers using the primers and annealing temperatures listed in Supplementary Table 2. Forward primers were labeled with 6-carboxy-fluorescine (6-FAM) (Schuelke, 2000) and PCR products were examined by capillary electrophoresis using the 3730 DNA Analyzer (Applied Biosystems) with a capillary length of 50 cm. Amplicon sizes were estimated with GeneMapper 4.0 (Applied Biosystems).

3. Results

The results of the PCRs are summarized in Table 1. As is usual when human aDNA is studied, there was greater success in amplifying the multicopy mtDNA markers compared with the two-copy amelogenin gene and autosomal STRs. Among the mtDNA results, there was consistency in terms of which samples gave PCR products, with all but one of the positive samples giving results with at least two different PCRs. These positive PCRs were reproducible with duplicate extracts, and independent replication in the second laboratory (Madrid) was successful for all five samples for which this was attempted.

Amelogenin PCRs were successful for 12 samples, but only four of these could be reproduced clearly with separate extracts. Of the 12 samples, seven were female and five male (Table 2). The four samples that gave reproducible results comprised two females and two males.

Fifteen of the samples yielded mtDNA sequence data but for one of these (AK3) the amount of data was insufficient for analysis. The other 14 samples revealed a variety of haplogroups with no two samples displaying identical sets of polymorphisms (Table 3). This conclusion is not affected by the missing data (amplicons that could not be obtained for particular samples, see Table 1), but is based on direct comparisons of amplicon sequences. For 12 samples there were sufficient polymorphisms for haplogroups to be assigned. Four samples (AK6, AK10, AK24, AK25) belonged to haplogroup H but with different HVR-I and HVR-II haplotypes. Similarly, AK23 and AK38 were both assigned to haplogroup T2, but with different polymorphisms that placed them in T2e and T2b, respectively. Two samples (AK5 and AK9ii) gave haplogroups identical to ones possessed by individuals who had handled the bones and/or were involved in the DNA analysis (Supplementary Table 3). From the available sequence data, the possibility that the AK5 and AK9ii results are due to contamination from these sources cannot be excluded.

The autosomal STR PCRs gave sporadic results but with a variety of alleles detected (Table 4). Six samples (AK5, AK6, AK17, AK18, AK23 and AK25) possessed CSF1PO alleles 119 and/or 123, indicating possible relationships. Similarly, D13S317 allele 133 was displayed by AK26, AK35 and AK38.

3. Discussion

3.1 Authenticity of the results

The difficulties involved in genetic analysis of ancient human skeletons are well known, especially the need to exclude modern contamination and to assess the authenticity of any ancient sequences that are obtained (Brown and Brown, 1992; Cooper and Poinar, 2000; Gilbert et al., 2005). Assessing the authenticity of DNA detections is particularly difficult with skeletal assemblages that were excavated in the past by individuals who are no longer available to donate DNA samples that can be tested as possible sources of contamination. We have shown, however, that most of the DNA contamination resulting from handling is located on the surface of a bone and can be eliminated by removing the outer 1–2 mm and then UV irradiating and treating with bleach (Bouwman et al., 2006), as we did in this project. These, and subsequent manipulations, were carried out in specially designed labs by personnel wearing full forensic clothing. We also included two types of control PCR, one to monitor for contamination of the materials used during DNA extraction and one to monitor contamination of the PCR reagents. None of the control PCRs yielded products of any size, suggesting that appropriate levels of cleanliness had been attained. All of the mtDNA sequences were replicated with separate extracts and those for five samples were independently replicated in a second laboratory. The 12 samples for which mtDNA haplogroups could be assigned each gave a different set of polymorphisms, each set consistent with a single mtDNA haplogroup, with no evidence that any individual sample contained DNA from two or more sources. If the mtDNA results are due entirely to

contamination then we must conclude that 12 different people independently contaminated a single bone, and no other bone, a scenario that seems incompatible with any reasonable assumption of the approach taken to the original excavation and osteological examination of these skeletons. The scenario is possible but we consider the alternative explanation, that these detected DNAs are ancient in origin, to be more likely. Nevertheless, based on comparisons between the mtDNA sequences obtained from the samples with the equivalent sequences of all living individuals who had handled the bones and/or were involved in the DNA analysis, we exclude the mtDNA results for two samples, AK5 and AK9ii, from our analyses as possible contaminants. We take this precaution even though the K haplogroup possessed by AK9ii is common in European populations (c.10% in modern Europeans), giving a high chance that the similarity between this sample and the lab personnel is purely coincidental.

3.2 Implications of the results for Romano-British infanticide

The autosomal STR results indicated two groups of infants (AK5, AK6, AK17, AK18, AK23 and AK25; and AK26, AK35 and AK38) each of which shared a variety of alleles in common. Whilst this would be compatible with a close genetic relationship between group members, because autosomal STRs in general show much less inter-individual variability than mtDNA, sharing of alleles between unrelated individuals is common. Therefore this sharing of alleles among the Hambleden infants may simply be a chance effect rather than evidence for close kinship. Osteological study (Mays et al., 2011) showed that two infants (AK22 and AK30) bear a supra-condyloid process on their humeri. This is a rare osteological variant that may have a genetic component in its causation, leading to the suggestion that they may share close kinship. Unfortunately, DNA amplification was unsuccessful in the samples from these burials.

In contrast with the STR results, the mtDNA indicated that no infants for which PCR was successful showed identical polymorphisms, showing that no two infants came from the same mother. It was hypothesised that because employees in a brothel would likely incur repeated unwanted pregnancies, some infants would have haplotypes in common. That this proved not to be the case may argue (albeit not very conclusively) against the brothel theory.

The amelogenin PCR resulted in the identification of sex in 12 infants, among which the male: female ratio was 0.7:1. This does not differ from the natural neonatal sex ratio of 1.05:1 (Hutchinson et al., 1963) (chi-square=0.44, ns). This contrasts with the bathhouse site at Roman Ashkelon, Israel, where study of the amelogenin gene identified an excess of males (Faerman et al., 1998). As discussed above, this pattern was interpreted as consistent with the function of that institution as a brothel. Whilst the balanced sex ratio among the Hambleden infants stands in contrast to the pattern seen at Ashkelon, it resembles that from other Romano-British sites. Mays & Faerman (2001) successfully identified sex using PCR of the amelogenin gene in 13 perinatal infants. These burials came from Romano-British burial sites which do not relate to brothels. However, like Hambleden, there was demographic evidence, in the form of perinatal age at death profiles strongly peaked at around full-term, for infanticide. There were nine males, four females, a sex ratio that does not differ from that expected naturally. Taken together, the current study, and that of Mays & Faerman (2001), have identified sex in a total of 25 perinatal infants from Romano-British sites for which there is evidence of infanticide; 14 were identified as male, 11 as female. Although numbers are small, the evidence currently available provides no support for the notion of infanticide specifically directed at females in Roman Britain. To some extent this finding appears to run contrary to written sources for female infanticide at that time. However this conflict may be more apparent than real. Although the written evidence for the Roman practice of infanticide is considerable (e.g.

Brunt, 1971; Langer, 1974; Wiedermann, 1989; Harris, 1994), that specifically for female-directed infanticide is less impressive, almost without exception relying on drama, novels and poetry for its sources, so that it is impossible to know how regularly it was practiced (Scheidel, 2010). In addition, the sources refer to Rome rather than provinces of the Empire such as Britain (Watts, 2001; Scheidel, 2010).

Turning to the high male: female ratios among adults in some Romano-British cemeteries, the current results provide no support for the contention that this reflects female-directed infanticide. Meta-analysis of cemetery demographic data (Davison, 2001) suggests that the sex imbalance is particularly associated with urban sites, so explanations involving migration of males to towns for military or occupational reasons may be important.

Conclusions

Analysis of the amelogenin gene among perinatal infant burials from Hambleden indicates a balanced sex ratio among those where DNA amplification was successful. This result stands in contrast to results from a brothel site in Roman Ashkelon, Israel, where there was an excess of males, a result that was interpreted as consistent with selective rearing of female offspring into the trade by courtesans (Faerman et al., 1998). mtDNA data indicate that at Hambleden all the infants were from different mothers. This too may, at least to some extent, argue against an existing interpretation of the Hambleden site as the location of a brothel as one might in that case have expected repeated unwanted pregnancies in individuals.

The current results add to a previous study (Mays & Faerman, 2001) using DNA to identify sex in Romano-British infants at sites where there is independent evidence for infanticide. That work, like the current study, indicated a balanced sex ratio among the infants. Results so far available from sex identification of perinatal infant remains provide

no evidence to support the notion that manipulation of the sex ratio was an important motivation for infanticide in Roman Britain.

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References

Andrews, R.M., Kubacka, I., Chinnery, P.F., Lightowlers, R.N., Turnbull, D.M., Howell, N., 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nature Genet 23, 147.

de la Bédoyère, G. 1993. Roman Villas and the Countryside. Batsford / English Heritage, London.

Bonsall, L. in press. Infanticide in the archaeological record. A critical review of the osteological evidence, with particular reference to Roman Britain. Childhood in the Past

Bouwman, A.S., Brown, T.A., 2005. The limits of biomolecular palaeopathology: ancient DNA cannot be used to study venereal syphilis. J Archaeol Sci 32, 691–702.

Bouwman, A.S., Chilvers, E.R., Brown, K.A., Brown, T.A., 2006. Identification of the authentic ancient DNA sequence in a human bone contaminated with modern DNA. Am J Phys Anthropol 131, 428–431.

Bouwman, A.S., Brown, K.A., Prag, A.J.N.W., Brown, T.A., 2008. Kinship between burials from Grave Circle B at Mycenae revealed by ancient DNA typing. J Archaeol Sci 35,

2580–2584.

Brown, T.A., Brown, K.A., 1992. Ancient DNA and the archaeologist. Antiquity 66, 10–23.

Brown, T., Brown, K. 2011. Biomolecular Archaeology: An Introduction. Wiley-Blackwell, Chichester.

Cooper, A., Poinar, H., 2000. Ancient DNA: do it right or not at all. Science 289, 1139.

Brunt, P.A. 1971. Italian Manpower 225BC – AD14. Clarendon, Oxford.

Cocks, A.H. 1921. A Romano-British homestead, in the Hambleden valley, Bucks. Archaeologia 71, 141-198.

Davison, C. 2001. Gender imbalances in Romano-British cemetery populations: a re-evaluation of the evidence. In (Pearce, J., Millett, M, Struck, M., eds) Burial, Society and Context in the Roman World. Oxbow, Oxford, pp. 231-237.

Divale, W.T., Harris, M. 1976. Population, warfare and the male supremacist complex. Am Anthropol 78, 521-538.

Eyers JE. 2011. Romans in the Hambleden Valley: Yewden Roman Villa. Chiltern Archaeology, High Wycombe.

Faerman, M., Filon, D., Kahila Bar Gal, G., Greenblatt, C.L., Stager, L., Oppenheim, A., Smith, P. 1998. Determination of the sex of infanticide victims from the late Roman era through DNA analysis. J Archaeol Sci 25, 861-865.

Frere, S. 1967. Britannia, a History of Roman Britain. Routledge & Keegan Paul, London.

Gilbert, M.T.P., Bandelt, H.-J., Hofreiter, M., Barnes, I., 2005. Assessing ancient DNA studies. Trends Ecol Evol 20, 541–544.

Hall, T.A.,1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.

Harris, W.V. 1982. The theoretical possibility of extensive infanticide in the Graeco-Roman world. Class Quart 32, 114-116.

Harris, W.V. 1994. Child exposure in the Roman Empire. J Roman Stud 84, 1-22.

Langer, W.L. 1974. Infanticide: a historical survey. Hist Child Quart 1(3): 353-365.

Lewis, M. 2007. The Bioarchaeology of Children: Perspectives from Biological and Forensic Anthropology. Cambridge University Press, Cambridge.

Lewis, M.E., Gowland, R. 2007. Brief and precarious lives: infant mortality in contrasting sites from Mediaeval and Post-Mediaeval England (AD850-1859). Am J Phys Anthropol 134, 117-129.

Lewis, N. 1985. Life in Egypt Under Roman Rule. Clarendon, Oxford.

Mays, S. 1993. Infanticide in Roman Britain. Antiquity 67, 883-888.

Mays, S. 1995. Child killing through the ages. Brit Archaeol 2, 8-9.

Mays, S. 2003. Comment on 'A Bayesian approach to ageing perinatal skeletal material from archaeological sites: implications for the evidence for infanticide in Roman Britain' by R.L. Gowland and A.T. Chamberlain. J Archaeol Sci 30, 1695-1700.

Mays, S. in press. The bioarchaeology of infant and child homicide. In (Thompson, J.L., Alfonso-Durruty, M.P. and Crandall, J.J., eds) Tracing Childhood: Bioarchaeological Investigation of Early Lives in Antiquity. Gainesville: University Press of Florida.

Mays, S., Cox, M. 2000. Sex determination in skeletal remains. In (M. Cox, S. Mays, eds) Human Osteology in Archaeology and Forensic Science. Cambridge: Cambridge University Press, pp. 117-130.

Mays, S., Eyers, J. 2011. Perinatal infant death at the Roman villa site at Hambleden, Buckinghamshire, England. J Archaeol Sci 38, 1931-1938.

Mays, S., Faerman M. 2001. Sex identification in some putative infanticide victims from Roman Britain using ancient DNA. J Archaeol Sci 28, 555-559.

Mays, S., Vincent, S., Robson-Brown, K., Roberts, A. 2011. The human remains. In (Eyers, J.E., ed) Romans in the Hambleden Valley: Yewden Roman Villa. High Wycombe: Chiltern Archaeology, pp. 247-258.

Philpott, R. 1991. Burial Practices in Roman Britain. A Survey of Grave Treatment and Furnishing AD43-410. British Archaeological Reports (British Series) 219. Tempus Reparatum, Oxford.

Saunders, S.R. 2008. Juvenile skeletons and growth-related studies. In (Katzenberg, M.A., Saunders, S.R., eds) Biological Anthropology of the Human Skeleton (2nd edition). Wiley-Liss, Chichesster, pp. 117-147.

Scheidel, W. 2010. Greco-Roman sex ratios and femicide in comparative perspective. Princeton / Stanford Working Papers in Classics. www.princeton.edu/~pswpc/pdfs/scheidel/o11003.pdf

Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnol 18, 233–234.

Smith, P., Kahila, G. 1992. Identification of infanticide in archaeological sites: a case study from the late Roman – early Byzantine periods at Ashkelon. J Archaeol Sci 19, 667-675.

Watts, D. J. 1989. Infant burials and Romano-British Christianity. Archaeol J 146, 372-383.

Watts, D.J. 2001. The silent minority: women in Romano-British cemeteries. Archaeol J 158, 332-347.

Wiedemann, T. 1989. Adults and Children in the Roman Empire. Routledge, London.

Table

Table 1

Results of PCRs.

Sample	Sex	mtDNA							Autosomal STRs							
	Amel	mtA	mtC	mtD	mtF	mtG	mtV	mtW	D8S1179	D3S1358	TPOX	D16S539	CSF1PO	TH01	D13S317	D5S818
AK2	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AK3	-	-	-	√√	-	-	-	-	-	-	-	-	-	-	-	-
AK4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AK5	-	-	√√	√√	√√	√√	-	√√	-	-	-	-	√	-	-	-
AK6*	-	-	√√	√√	-	√√	-	√√	-	-	-	-	√	-	√	-
AK7	-	-	√√	√√	-	√√	-	√√	-	-	-	-	-	-	-	-
AK8	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AK9i	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AK9ii	-	-	√√	-	√√	-	-	√√	-	-	-	-	-	-	-	-
AK10	√	-	√√	√√	√√	√	-	√√	-	-	-	-	-	-	-	-
AK12	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

AK13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AK14	-	-	√√	√√	√√	-	-	√√	-	-	-	-	-	-	-	-
AK15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AK16	√	-	-	-	-	-	-	-	-	-	-	-	√	-	√	-
AK17*	-	-	√√	√√	√√	-	-	-	-	-	-	-	√	-	-	-
AK18	-	-	-	-	-	-	-	-	-	-	-	-	√	-	-	-
AK20	√	-	-	-	-	-	-	-	√	-	-	-	√	-	-	-
AK22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AK23	-	-	-	√√	√√	-	-	-	-	-	-	-	√	-	-	-
AK24	-	-	√√	√√	√√	-	-	-	-	-	-	-	-	-	-	-
AK25	-	-	√√	√√	√√	√√	-	-	-	-	-	-	√	-	-	-
AK26*	-	-	-	√√	√√	√√	-	-	-	-	-	-	-	-	-	-
AK27	√√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AK28	√√	-	-	-	-	-	-	-	-	-	-	-	-	-	√	-
AK30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AK31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AK35	-	-	-	-	-	-	-	-	√	-	-	-	-	-	√	-
AK38*	√	-	√√	√√	√√	√√	-	√√	-	-	-	-	√	-	√	-

AK40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AK42*	√√	√√	√√	√√	√√	-	-	√√	-	-	-	-	-	-	-	-
AK43	√√	√√	√√	√√	√√	-	-	√√	-	-	-	-	-	-	-	-
AK45ii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: - no successful PCRs; √, one successful PCR; √√, two successful PCRs.

*MtDNA results for AK6, AK17, AK26, AK38 and AK42 were replicated in Madrid.

Table 2

Results of DNA-based sex identifications.

Sample	Sex
AK2	Male
AK8	Female
AK9i	Female
AK10	Female
AK12	Female
AK16	Male
AK20	Male
AK27	Female
AK28	Female
AK38	Female
AK42	Male
AK43	Male

Table 3

Mitochondrial DNA sequencing results and haplogroup assignments.

Sample	Polymorphisms compared with rCRS*		Deduced haplogroup
	HVR-I	HVR-II	
AK5	16343G, 16390A	73G, 263G, 315.1C	U3
AK6	16354T	263G, 315.1C	H
AK7	16129A	263G, 315.1C	Unassigned
AK9ii	16224C, 16311C	73G, 263G	K
AK10	16085T, 16104T	73G, 263G, 315.1C	H
AK14	16294T	No amplicons	Unassigned
AK17	16105C, 16189C, 16223T, 16278T	263G, 315.1C	X
AK23	16126C, 16153A, 16294T	No polymorphisms	T2e
AK24	16104T, 16224C	73G	H3n
AK25	16294T	263G, 315.2C	H42a
AK26	16298C, 16395T, 16129A	263G, 309.1C, 315.1C	V

AK38	16294T, 16296T, 16304C	73G, 263G, 309.1C, 315.1C	T2b
AK42	16069T, 16172C, 16222T, 16261T	73G	J
AK43	16256T, 16270T, 16298C, 16306T, 16366T, 16367T	72C	U5a

*rCRS, revised Cambridge Reference Sequence (Andrews et al., 1999). The polymorphisms listed for hypervariable regions (HVR) I and II are those located within the amplicons that were obtained; as the entire HVR was not amplified in each specimen, some additional polymorphisms might be present in unsequenced regions.

Table 4

Results of autosomal STR typing.

Sample	Alleles detected*		
	CSF1PO	D8S1179	D13S317
AK5	119	-	-
AK6	123	-	120, 124
AK16	114, 118	-	129
AK17	119, 123	-	-
AK18	119	-	-
AK20	170	96	-
AK23	119, 123	-	-
AK25	119, 123	-	-
AK28	-	-	133
AK35	-	109	133
AK38	127	-	133

*Numbers refer to the sizes of the amplicons in bp.

Supplementary Material

Click here to download Supplementary Material: SI.docx