



# Sex Identification in Some Putative Infanticide Victims from Roman Britain Using Ancient DNA

Simon Mays

*Ancient Monuments Laboratory, English Heritage Centre for Archaeology, Fort Cumberland, Fort Cumberland Road, Eastney, Portsmouth PO4 9LD, U.K.*

Marina Faerman

*Laboratory of Biological Anthropology and Ancient DNA, Hadassah School of Dental Medicine, Hebrew University, PO Box 12272, 91120 Jerusalem, Israel*

*(Received 15 May 1999, revised manuscript accepted 3 August 2000)*

Previous study of infant burials has suggested that infanticide was routinely practised during the Roman period in Britain. This, together with the observation that there is an adult sex imbalance in favour of males at many Romano-British cemetery sites, has raised the question of female infanticide. We attempted to investigate this possibility by identifying sex in some infant skeletons from Romano-British contexts using ancient DNA (aDNA) techniques. Of 31 individuals sampled, sex identification was successful in 13, of which nine were males and four females. These results are discussed in the light of previous work on DNA-based sexing of infant burials.

© 2001 Academic Press

*Keywords:* ANCIENT DNA, SEX IDENTIFICATION, INFANTICIDE, ROMAN BRITAIN.

## Introduction

Infanticide, the killing of unwanted babies, has been practised on all continents and at every level of social complexity (Williamson, 1978). Infanticide has received much attention from anthropologists (e.g. papers in Hausfater & Hrdy, 1984) and historians (e.g. Hoffer & Hull, 1981; Harris, 1982), but it has been less well studied archaeologically. This stems in part from problems in identifying the practice in the archaeological record. However, recent work has suggested that it is possible to infer whether infanticide was practised on a regular basis in earlier populations by demographic analysis of age at death of perinatal burials. Specifically, a perinatal age distribution showing a strong peak at an age approximately corresponding to full term is suggestive of infanticide, given that the deed is generally carried out at about time of birth; natural deaths give a rather flatter age distribution (Mays, 1993; Smith & Kahila, 1992). In Britain, it has been demonstrated (Mays, 1993) that age at death distributions of perinatal infants from the Roman period show a strong peak at about 38–41 weeks (approximately the gestational age of a full term baby). By contrast, Mediaeval infants show a much flatter age distribution, with no strong peak. It was argued that this suggested infanticide in Roman times, whilst the

Mediaeval infants were likely natural deaths. In Israel, Smith & Kahila (1992) studied infant remains from a Roman sewer, which ran beneath a bathhouse in Ashkelon. From long-bone size, dental development and lack of neonatal lines in the teeth, they deduced that most infants died at around birth, and this, together with the fact that these individuals were disposed of in the sewer rather than given regular burial, was interpreted as evidence for infanticide.

In many societies infants of one sex are valued more than the other. It is usually male offspring which are preferred. When such societies practise infanticide, more girls than boys are likely to be victims (Nordborg, 1992). That infanticide existed in Classical Rome is well established from documentary sources (Langer, 1974), and a variety of motives for it are recorded, among which are that the child was not of the desired sex. It is clear that this normally meant that the child was a girl (Wiedemann, 1989: 37; Lewis, 1985: 54), so more female babies may have been victims of infanticide. In this context it is of interest to note that most cemeteries in Britain, dating from the period when it was a province of the Roman Empire, show imbalanced adult sex ratios. Combined data from about 2400 adult burials from a number of large burial grounds reveal a sex ratio of 1.46:1 in favour of males (Mays, 1995). Although a number of different factors

might account for this pattern, female infanticide is one possible explanation.

In the light of the evidence, both for infanticide and for a biased adult sex ratio in Roman Britain, it would be of value to investigate the possibility of female infanticide by sexing Roman infant burials. Existing morphological methods do not permit the reliable identification of sex in this class of human skeletal remains (Mays & Cox, 2000). However, a number of studies have demonstrated the value of DNA analysis for sex identification in early skeletons (Faerman *et al.*, 1995, 1998; Stone *et al.*, 1996; Lassen *et al.*, 1996; Evison, 1999; Vernesi *et al.*, 1999; discussion in Brown, 1998). The present work is an attempt to perform DNA-based sex identification in some Romano-British infants from sites where infanticide has previously been inferred.

## Materials and Methods

The skeletal material used in the present work comes from two Romano-British burial sites, Ancaster, Lincolnshire (Cox, 1989) and Thistleton, Rutland (Powers *et al.*, nd; Mays, nd). These sites formed part of the combined Romano-British data set for which previous work (Mays, 1993) has shown that the age at death distribution suggested infanticide. Bones from a total of 31 individuals (13 from Ancaster, 18 from Thistleton) were subjected to DNA analysis. For these individuals, long-bone lengths were measured and age at death estimated from them using the linear regression equations of Scheuer, Musgrave & Evans (1980).

DNA was isolated from bone powder obtained from the infant long bones. For each bone specimen, two separate DNA extracts were obtained and analysed several months apart with newly prepared solutions. The external layer of bones was removed with a sterile scalpel blade. Bone powder was obtained by scraping the uncovered surface with a new sterile blade. DNA was isolated from 5–25 mg of bone powder, following silica-based purification protocol (Höss & Pääbo, 1993).

Sex identification was performed using a method based on PCR amplification of the X and Y amelogenin alleles. The reaction yields distinguishable PCR products of the X and Y amelogenin alleles by the simultaneous use of three primers (Faerman *et al.*, 1995). The primers M4, M5 and M6 amplify PCR products of 329-bp (X allele) and 235-bp (Y allele), and not 330-bp and 218-bp as was incorrectly reported previously (Faerman *et al.*, 1995). The 5' primer (M4 5'-CAGCTTCCAGTTTAAGCTCT-3') is common to both the X and Y amelogenin alleles, while the 3' primers (M5 5'-TCTCCTATACCACTTAGTCACT-3' and M6 5'-GCCCAAAGTTAGTAATTTTACCT-3') are specific for one or other. The detection limit for the X allele is 25 pg and for the Y allele 5–10 pg in modern DNA after 35 cycles.

In the present study PCR conditions were as described previously (Faerman *et al.*, 1995) except that 50 cycles of amplification were performed. From 5 to 10 µl of each aDNA extract was subjected to hot-start PCR amplification in a 25 µl reaction. A minimum of four PCR reactions were performed for each specimen. In part of the experiments, the upstream primer M4 was replaced by 5'-TCATGAACCACTGCTCAGG-3' described in Götherström *et al.* (1997). This combination of primers results in shorter amplification products of the X (214-bp) and Y (119-bp) amelogenin alleles. PCR conditions remained the same except that the MgCl<sub>2</sub> concentration was decreased to 1.5 mM (final) and the annealing step was performed at 53°C.

Twenty-microlitre aliquots of the PCR products were analysed on 2% Nusieve agarose gel stained with ethidium bromide. To verify the authenticity of the X and Y amelogenin alleles, the respective bands were isolated from the gels with a sterile Pasteur pipette and 3–5 µl was used for direct <sup>33</sup>P terminator cycle sequencing (Amersham Life Sciences Inc., U.S.A.) with allele-specific (M5 and M6) primers.

Stringent precautions were undertaken to eliminate contamination by DNA from exogenous sources. These included physical separation of work areas from those used for analysis of modern DNA and separation of experimental steps: DNA extraction and PCR set up were performed in two different, daily UV-irradiated hoods, located in different rooms separated from the analysis of DNA products. Ultra-pure reagents dedicated to aDNA analysis only, sterile disposable plastic ware and filtered tips were used, while masks, gloves and laboratory coats were worn. Appropriate negative controls were included for each step of the procedure: blank extraction controls without bone powder and blank PCR controls containing water instead of aDNA extract.

## Results

Of the 31 specimens, sex identification was achieved in 13 (six from Ancaster, seven from Thistleton). The results are summarized in Table 1. Altogether nine specimens were identified as male (four from Ancaster, five from Thistleton), four as female (two from each site). There were no inconsistent findings in the two independent aDNA extracts for any of the specimens. For two individuals from Ancaster two different bones were available for DNA analysis. Burial A48 was identified as male by the amplification of the 235-bp Y amelogenin allele in two independent DNA extracts obtained from the femur and the humerus. The male sex of specimen A153 was confirmed in two independent aDNA extractions from the femur and the tibia.

Using M4, M5 and M6 primers, that amplify the 329-bp X and the 235-bp Y amelogenin alleles, we could identify sex of 11 infants: nine males and two females. Importantly, only the Y amelogenin allele

Table 1. Results of DNA-based sex identification in Romano-British infants

Burial	Bone(s) sampled	PCR results	Sex
A48	Femur and humerus	Y	Male
A90	Humerus	X*	Female
A95	Femur	Y	Male
A101	Tibia	X	Female
A114	Tibia	X,Y*	Male
A153	Femur and tibia	Y	Male
THV132	Tibia	X,Y*	Male
THV135	Tibia	Y	Male
THZ2184	Femur	X*	Female
THZ2185	Femur	Y	Male
THZ2268	Humerus	X	Female
THZ2712	Humerus	Y	Male
THZ2715	Ulna	Y	Male

Note: Burials from Ancaster prefixed with A, burials from Thistleton prefixed with TH.

\*In part of the experiments the primer M4 was replaced with the upstream primer described in Götherström *et al.* (1997), thus resulting in the amplification the 214-bp and 119-bp long PCR products of the X and the Y amelogenin alleles, respectively.

could be amplified in all nine male samples, thus reflecting a relatively poor state of DNA preservation in the bone specimens. Moreover, previously we reported that in our experiments with modern DNA we had observed preferential amplification of the Y allele when less than 25 µg of DNA was used per reaction (Faerman *et al.*, 1995). To avoid the possibility of missing females in degraded aDNA samples, the primer M4 was replaced by the upstream primer designed by Götherström *et al.* (1997). This new combination of primers yields shorter amplification products of 214 and 119 bp of the X and Y amelogenin alleles respectively. For those aDNA extracts which had not yielded PCR results, amplification was repeated with a new set of primers (Figure 1). In addition, the A90 and THZ2184 specimens were identified as females by the amplification of the 214-bp X amelogenin allele. In specimens A114 and THV132 both X (214-bp) and Y (119-bp) amelogenin alleles were amplified. It is worth mentioning that using the first set of primers we were able to amplify only the Y (235-bp) amelogenin allele in these two samples. The authenticity of the amplified fragments was verified by direct sequencing of the respective bands of the four male and female specimens shown in Figure 1.

No amplification products were obtained in 4 PCR reactions for each of the remaining 18 bone specimens, a total of 72 PCR reactions. Furthermore, additional 25 cycles of amplification using 5 µl aliquots of the first round PCR did not provide any results (a total of 144 PCR reactions).

The long-bone length and age at death data for the 13 individuals for whom sex was identified based on molecular findings is given in Table 2. Comparison with modern data (O'Brien & Queenan, 1981; Hohler & Quetel, 1982; Yeh *et al.*, 1982) shows that the

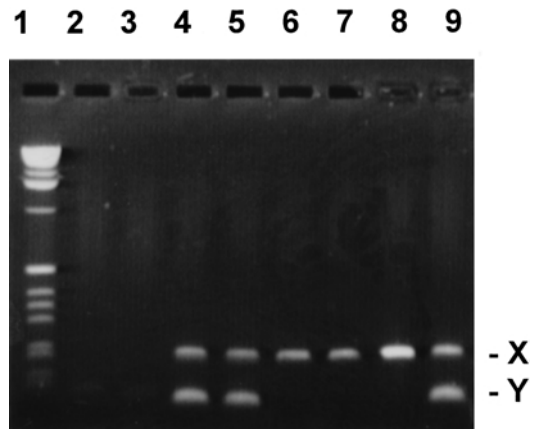


Figure 1. PCR amplification of the X (214-bp) and Y (119-bp) amelogenin alleles in DNA samples recovered from the infant long bones. Lane 1, 1 kb DNA size marker; lane 2, "no DNA" PCR control; lane 3, "no DNA" extraction control; lane 4, A114; lane 5, THV 132; lane 6, A90; lane 7, THZ2184; lane 8, modern female DNA control; lane 9, modern male DNA control.

Table 2. Long-bone length and ageing data for the infants whose sex was identified using aDNA

Burial	Femur length*	Humerus length	Ulna length	Estimated age
A48	75	—	—	38
A90	83	—	—	41
A95	—	—	—	40†
A101	80	—	—	40
A114	83	—	—	41
A153	—	—	54	35
THV132	77	68	—	39
THV135	78	69	—	39
THZ2184	76	68	61	39
THZ2185	78	—	—	39
THZ2268	75	65	—	38
THZ2712	76	—	62	39
THZ2715	—	69	63	40

Note: Burials from Ancaster prefixed with A, burials from Thistleton prefixed with TH.

\*Diaphysal lengths of femur, humerus and ulna in mm are from Powers *et al.* (nd) and Cox (1989). Estimated gestational ages in weeks are derived from long-bone diaphysal lengths using the linear regression equations of Scheuer, Musgrave & Evans (1980), their femur length equation being used when this measurement is available.

†For this burial long-bone diaphyses are too eroded to permit precise length measurements, however visual comparison of bone sizes with the other burials clearly indicates an age at death of about 40 weeks.

long-bone lengths are as expected if these infants died at around time of birth. Consistent with this, the age estimations from Scheuer, Musgrave & Evans (1980) regression equations cluster around 38–41 weeks, corresponding to about a full-term infant (Tanner, 1989: 43). In this respect the results are similar to those from the composite Romano-British assemblage (Mays, 1993) of perinatal infants of which these 13 burials form a sub-set. It is clear that the long-bone length and

the estimated age at death data fulfil the criteria of Smith & Kahila (1992) and Mays (1993) for the presence of infanticide.

## Discussion

The sex ratio in the present study material is 2.25:1 in favour of males. Because of the fairly small number of individuals which we were able successfully to sex, the sex imbalance in the present material does not differ significantly from the natural neonatal sex ratio of 1.05:1 (Cowgill & Hutchinson, 1963) (chi-square=1.7, ns). The present results offer no support for the hypothesis of excess female perinatal deaths, and hence, if one accepts the infanticide thesis, for female-directed infanticide. However, the numbers are too small to come to any very firm conclusions. Sample size was reduced by poor DNA survival, which permitted successful DNA amplification in only 42% of cases. This success rate is a little on the low side compared with that reported in most published literature. Previous investigators have given figures of between 44% and 85% successful for amelogenin-based DNA sex identification in archaeological human remains (Brown, 2000: Table 2).

In the present study 235-bp long DNA fragments of the Y allele were consistently amplified in nine male specimens. No additional males were identified when a second set of primers was applied decreasing the size of the Y fragment to only 119-bp. This finding suggests that our relatively low identification success rate could not be due to the large size of the DNA fragments required for the test used here. Furthermore, our results showed that when the M4 primer was replaced by that of Götherström *et al.* (1997), decreasing the size of the X allele fragment from 329-bp to 214-bp, only two out of 20 (10%) previously unidentified bone samples could be sexed. The 214-bp X allele fragment of the second test is even shorter than the 235-bp Y fragment of the first test, which in this study resulted in positive identification of nine male specimens. These findings suggest that it is unlikely that we have significantly underestimated the number of females.

In the only other attempt to identify sex in Romano-British infants using aDNA of which we are aware, Waldron, Taylor & Rudling (1999) tried to amplify amelogenin sequences in the DNA extracts from seven perinatal Roman infant burials from Sussex. Sex was successfully identified in four cases, of which three were male. As with the present work, their sample was too small to come to firm conclusions concerning whether there was an excess deaths of one sex. However, as with our results, they indicate that if their infants are accepted as infanticide victims then the practice was clearly not confined to one sex. Combining our own and Waldron, Taylor & Rudling's (1999) results gives a total of 12 males, 5 females. Although the difference between this and the natural sex ratio at birth of 1.05:1

fails to attain conventional levels of statistical significance (chi-square=2.6,  $P=0.11$ ) it is perhaps suggestive of an over-representation of male deaths among Romano-British infants. Interestingly, Faerman *et al.* (1998) successfully sexed some of the Ashkelon babies and also found an excess of males. They connected this with the specific nature of that site: bathhouses often functioned as brothels, and courtesans may have selectively reared some offspring (mostly females) into their profession, discarding the others.

Given the difficulties we have experienced in obtaining amplifiable nuclear DNA from these burials, our study cannot provide firm conclusions regarding the preferential infanticide of one sex or other in Roman Britain. However, it does add to the corpus of data of sexed infant burials from this period. The relatively low rate of successful DNA amplification in the present study should not discourage future workers from undertaking this type of work on Romano-British infants. Indeed, only by more such studies will a corpus of data accumulate sufficient to provide firm evidence concerning the question of sex-bias in infant deaths in Roman Britain.

## Acknowledgements

Thanks are due to Mike Taylor for permitting access to the results from his group for the Sussex Roman infant burials in advance of publication. We are grateful to Patricia Smith for support and fruitful discussion. We thank anonymous reviewers for critical reading of the manuscript. The work was supported by a research award from the British Academy.

## References

- Brown, K. (1998). Gender and sex—what can ancient DNA tell us? *Ancient Biomolecules* **2**, 3–15.
- Brown, K. (2000). Ancient DNA applications in human osteoarchaeology: achievements, problems and potential. In (M. Cox & S. Mays, Eds) *Human Osteology in Archaeology and Forensic Science*. London: Greenwich Medical Media, pp. 455–473.
- Cowgill, U. M. & Hutchinson, G. E. (1963). Differential mortality among the sexes in childhood and its possible significance in human evolution. *Proceedings of the National Academy of Sciences of the USA* **49**, 425–429.
- Cox, M. (1989). *The Human Bones From Ancaster*. London: English Heritage Ancient Monuments Laboratory Report 93/89.
- Evison, M. P. (1999). Ancient HLA: a preliminary survey. *Ancient Biomolecules* **3**, 1–28.
- Faerman, M., Filon, D., Kahila, G., Greenblatt, C. L., Smith, P. & Oppenheim, A. (1995). Sex identification of archaeological human remains based on amplification of the X and Y amelogenin alleles. *Gene* **167**, 327–332.
- Faerman, M., Kahila Bar-Gal, G., Filon, D., 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. *Journal of Archaeological Science* **25**, 861–865.
- Götherström, A., Liden, K., Ahlström, T., Kallersjö, M. & Brown, T. A. (1997). Osteology, DNA and sex identification: morphological and molecular sex identifications of five neolithic individuals from Ajvide, Gotland. *International Journal of Osteoarchaeology* **7**, 71–81.

- Harris, W. V. (1982). The theoretical possibility of extensive infanticide in the Graeco-Roman world. *Classical Quarterly* **32**, 114–116.
- Hausfater, G. & Hrdy, S. B. (1984). *Infanticide*. Hawthorne: Aldine.
- Hoffer, P. C. & Hull, N. E. H. (1981). *Murdering Mothers: Infanticide in England and New England 1558–1803*. New York: New York University Press.
- Hohler, C. W. & Quetel, T. A. (1982). Fetal femur length: equations for computer calculation of gestational age from ultrasound measurements. *American Journal of Obstetrics and Gynaecology* **143**, 479–481.
- Höss, M. & Pääbo, S. (1993). DNA extraction from Pleistocene bones by a silica-based purification method. *Nucleic Acids Research* **21**, 3913–3914.
- Langer, W. L. (1974). Infanticide: a historical survey. *History of Childhood Quarterly* **1**, 353–365.
- Lassen, C., Hummel, S. & Herrmann, B. (1996). PCR based sex identification of ancient human bones by amplification of X- and Y-chromosomal sequences: a comparison. *Ancient Biomolecules* **1**, 25–34.
- Lewis, N. (1985). *Life in Egypt Under Roman Rule*. Oxford: Clarendon.
- Mays, S. (1993). Infanticide in Roman Britain. *Antiquity* **67**, 883–888.
- Mays, S. (1995). Child killing through the ages. *British Archaeology* **2**, 8–9.
- Mays, S. (nd). The Burials from Thistleton, Leicestershire. Portsmouth: Report held on File at the Ancient Monuments Laboratory, English Heritage.
- Mays, S. & Cox, M. (2000). Sex determination in skeletal remains. In (M. Cox & S. Mays, Eds) *Human Osteology in Archaeology and Forensic Science*. London: Greenwich Medical Media, pp. 117–130.
- Nordborg, M. (1992). Female infanticide and human sex ratio evolution. *Journal of Theoretical Biology* **158**, 195–198.
- O'Brien, G. D. & Queenan, J. T. (1981). Growth of the ultrasound fetal femur length during normal pregnancy. *American Journal of Obstetrics and Gynaecology* **141**, 833–837.
- Powers, R., Cullen, R. & Jarman, S. (nd). Notes on the Human Bones from Thistleton. Portsmouth: Report held on File at the Ancient Monuments Laboratory, English Heritage.
- Scheuer, J. L., Musgrave, J. H. & Evans, S. P. (1980). Estimation of perinatal and late foetal age from limb bone length by linear and logarithmic regression. *Annals of Human Biology* **7**, 257–265.
- Smith, P. & Kahila, G. (1992). Identification of infanticide in archaeological sites: a case study from the Late Roman–Early Byzantine periods at Ashkelon, Israel. *Journal of Archaeological Science* **19**, 667–675.
- Stone, A. C., Milner, G. R., Pääbo, S. & Stoneking, M. (1996). Sex determination of ancient human skeletons using DNA. *American Journal of Physical Anthropology* **99**, 231–238.
- Tanner, J. M. (1989). *Foetus Into Man* (2nd edn). Ware: Castlemead.
- Vernesì, C., Caramelli, D., Carbonelli, S., Ubaldi, M., Rollo, F. & Chiarelli, B. (1999). Application of DNA sex tests to bone specimens from two Etruscan (VII–VIII Century BC) archaeological sites. *Ancient Biomolecules* **2**, 295–305.
- Waldron, T., Taylor, G. M. & Rudling, D. (1999). Sexing of Romano-British baby burials from the Beddingham and Bignor Villas. *Sussex Archaeological Collections* **137**, 71–79.
- Wiedemann, T. (1989). *Adults and Children in the Roman Empire*. London: Routledge.
- Williamson, L. (1978). Infanticide: an anthropological analysis. In (M. Kohl, Ed.) *Infanticide and the Value of Life*. Buffalo: Prometheus, pp. 61–75.
- Yeh, M-N., Bracero, L., Reilly, K. B., Murtha, L., Aboulafia, M. & Barron, B. A. (1982). Ultrasonic measurement of the femur length as an index of fetal gestational age. *American Journal of Obstetrics and Gynaecology* **144**, 519–522.