



Determining the Sex of Infanticide Victims from the Late Roman Era through Ancient DNA Analysis

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Infanticide has since time immemorial been an accepted practice for disposing of unwanted infants. Archaeological evidence for infanticide was obtained in Ashkelon, where skeletal remains of some 100 neonates were discovered in a sewer, beneath a Roman bathhouse, which might have also served as a brothel. Written sources indicate that in ancient Roman society infanticide, especially of females, was commonly practised, but that females were occasionally saved and reared as courtesans. We performed DNA-based sex identification of the infant remains. Out of 43 left femurs tested 19 specimens provided results: 14 were found to be males and 5 females. The high frequency of males suggests selective preservation of females and that the infants may have been offspring of courtesans, serving in the bathhouse, supporting its use as a brothel.

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Introduction

Today archaeologists are paying increasing attention to examining social structure within past societies. While gender differences have been traditionally explored through identification of

grave goods considered indicative of female or male roles, physical anthropology enables archaeologists to develop the study of mortuary practices through identification of gender in relation to burial type, even when grave goods are absent. However, the problem of sex determination remains

in dealing with fragmentary and/or infant burials. The reliability of morphometric analyses for gender identification in infants is low, especially in the case of incomplete skeletons.

Infant burials from the Neolithic to recent periods frequently occur in Israel in different archaeological contexts from those of older children or adults. These infant remains may have been treated with great care, as for example, the jar burials with grave goods found at Middle Bronze Age Kabri (Kempinski & Niemeier, 1992), or alternatively treated with complete disregard like the infants thrown into rubbish pits at Chalcolithic Shiqmim (Levy *et al.*, 1991), or into sewers in Late Roman Ashkelon (Stager, 1991; Smith & Kahila, 1992). Knowing the gender of infants found in different archaeological contexts has implications not only for the type of burial accorded, but also the possible role of gender in relation to the question of infant sacrifices and infanticide.

Human settlement of Ashkelon dates back over 5000 years, and during most of this period Ashkelon was a major seaport (Stager, 1993). It served the Canaanites from c. 2000–1200 BC, and was one of five main centres of the Philistines until 604 BC. Under Persian hegemony Phoenicians from Tyre colonized the seaport from 525–300 BC. They, in turn, were successively replaced by the Jews, the Greeks and finally the Romans in the 1st century AD.

Skeletal remains of more than 100 neonates were found during archaeological excavations by the Leon Levy Expedition to Ashkelon (Stager, 1991; Smith & Kahila, 1992; Faerman *et al.*, 1997). The infant remains were found in the sewer beneath a bathhouse, built in the 4th century and used until the 6th century. The infant bones had been discarded in the gutter of the sewer along with animal bones, potsherds and isolated coins; no signs of careful burial or associated grave goods were observed. The casual method of disposal contrasts sharply with the careful infant jar burial from the same period discovered some 200 yards away. Bone size, dental development and lack of neonatal lines in the teeth indicated that they were all neonates, 1–2 days old. The combination of early death of so many infants and their mode of disposal implied infanticide, rather than death from natural causes (Smith & Kahila, 1992). None of the infants showed evidence of disease or skeletal malformation, indicating that other factors, such as their gender, may have been the motive for infanticide.

New developments in molecular biology, and especially in analysing DNA recovered from ancient bones, have provided reliable methods for gender determination based on amplification of DNA sequences specific to the X and/or Y chromosomes (Gill *et al.*, 1994; Faerman *et al.*, 1995; Lassen, Hummel & Herrmann, 1996; Stone *et al.*, 1996).

This paper brings together material previously discussed by Smith & Kahila (1992) and Faerman *et al.* (1997).



Figure 1. Left femurs of Ashkelon infants. Scale is 1 cm.

Materials and Methods

DNA was isolated from the bone powder, obtained from left femurs only, to avoid testing the same individual twice (Figure 1). Altogether 43 left femurs were available for the analysis (29 complete and 14 fragmentary). Bones were cleaned with a soft brush. The surface layer was removed by electric drill (large bit), and bone powder was obtained by drilling in a freshly uncovered surface with a sterile small burr. Approximately 0.5–1.0 mg of bone powder was used for each DNA extraction. DNA from each specimen was extracted twice following the chelex purification procedure (Woodward *et al.*, 1994). A third extraction was performed and analysed at least 6 months later using a silica-based purification method (Hoss & Paabo, 1993).

Ancient DNA studies are prone to numerous artefacts (Paabo, Gifford & Wilson, 1988; Hagelberg & Clegg, 1991). To eliminate contamination by DNA of exogenous sources, stringent precautions were included at every step. Disposable sterile tubes, filtered tips and aliquoted sterile reagents and solutions, kept only for ancient DNA work, were used throughout. DNA extraction and polymerase chain reaction (PCR) were performed in different hoods, sterilized by UV light, and located in two different rooms. Different sets of pipettes were used for DNA extraction, PCR amplification and analyses of the PCR products. Blank extraction controls, containing no bone material, were run in parallel with each set of experiments.

We have applied a highly sensitive method based on PCR amplification of the X and Y amelogenin alleles (Faerman *et al.*, 1995). The reaction yields distinguishable X- and Y-chromosome products by the simultaneous use of three primers (Figure 2(a)). We subjected 3, 7 and 11 µl of each DNA extract to PCR amplification along with a blank extraction control and no DNA PCR control to monitor contamination during the DNA extraction and PCR amplification. Conditions for the PCR and the three primers (M4, M5 and M6) have been described previously (Faerman *et al.*, 1995). Primer M7 (5'-GTGACTATCTTAGA ATCAGGAG-3'), designed during this study (see

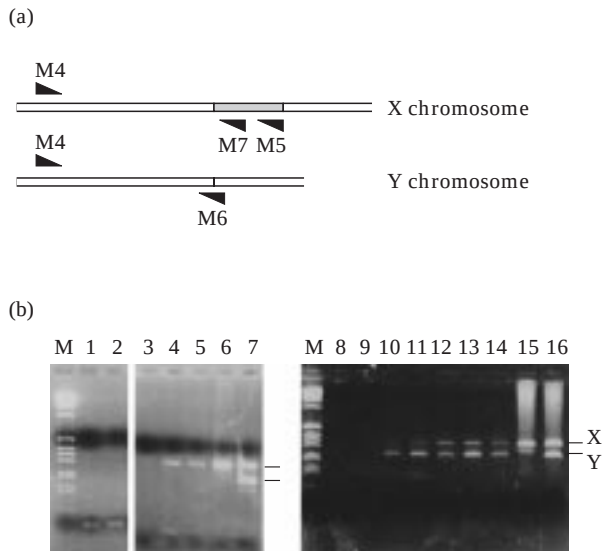


Figure 2. (a) Part of the amelogenin encoding gene showing the location of the PCR primers. Fragment deleted in the Y chromosome is indicated. (b) Sex identification of Ashkelon infants: M—size marker (1 kb DNA ladder); lanes 1, 8, “no DNA” PCR control; lane 2, “blank extraction” control; lanes 3–5, specimen No. 100; lanes 9–11, specimen No. 94; lanes 12–14, specimen No. 96; positive controls: lanes 6, 15, modern female DNA; lanes 7, 16, modern male DNA.

Results), was used in part of the experiments instead of primer M5. We analysed 18 μ l aliquots on 2% Nusieve agarose gel, stained with ethidium bromide. To verify the authenticity of the X and Y amelogenin alleles, the respective bands were sequenced. For this purpose 5 μ l of the PCR products along with the appropriate controls were subjected to additional 25 cycles, and the re-PCR products were purified by electrophoresis on 1% low melting agarose gel (Filon *et al.*, 1995). Sequence analysis was performed using the allele-specific primers with Sequenase Version 2.0 (USB).

Results

Amplification was successful for 19 out of the 43 ancient specimens tested. Fourteen specimens were found to be males and five females, giving a significantly higher frequency of boys than girls ($P < 0.05$). The results for three specimens are shown in Figure 2(b). The success rates of the PCRs are given in Table 1. In total, data were obtained for 70 of 189 PCRs of the 19 specimens. There were no inconsistencies or conflicting data for any of the specimens. Furthermore, the results for all the specimens, except specimen No. 107, were reproduced on at least two separate DNA extracts. The authenticity of the amplified fragments was verified by direct sequencing of the respective bands of male and female samples (not shown).

Successful amplification was obtained for 44% of the specimens examined, despite the antiquity and

Table 1. Data on DNA-based sex identification of the Ashkelon infants

Specimen	Successful PCRs*			Results
	Extract 1	Extract 2	Extract 3	
90	1	0	1	Male
91	1	0	1	Male
92	3	2	2	Male
93	2	2	0**	Male
94	2	2	2	Male
95	3	2	1	Male
96	2	3	1	Male
97	2	1	0**	Male
98	2	3	0**	Male
100	2	1	1	Female
104	1	1	0**	Male
107	2	0	0**	Female
119	2	0	2	Female
120	1	0	1	Female
122	1	2	2	Male
123	1	1	2	Male
216	1	1	0**	Female
221	1	1	0	Male
234	1	1	0	Male

*3 PCRs were performed for each DNA extract.

**6 PCRs were performed for each of these DNA extracts.

friable condition of the bones, and these included a significantly larger number of males than females.

In our experiments with contemporary DNA we have noticed preferential amplification of the Y allele when less than 25 pg DNA was used per reaction (Faerman *et al.*, 1995). We considered the possibility that for some reasons (difference in length of the X- and Y-specific PCR products, or nature of the primers) our test may miss females in highly degraded DNA specimens. We therefore designed a new X-specific primer, which together with the 5' common primer spans a smaller fragment (270 bp) than that previously used (see Figure 2(a)). For the 24 bone specimens which had not yielded amplifiable DNA, all tests were repeated in full, including DNA extraction and PCR amplification, with the new set of primers. No amplification products were obtained in three PCRs for each of two DNA extractions of each bone specimen, a total of 144 PCRs.

Discussion

The initial reasons for deciding that the individuals analysed here were victims of infanticide were the lack of infants aged more than 2 days and their casual disposal in the sewer (Smith & Kahila, 1992). If the Ashkelon sewer served as a public place for disposal of infants who had died naturally, but were considered too unimportant or too young for full burial rites, then one would expect to find infants of up to at least 3 months of age. Full burial rites were apparently rarely carried out for infants of less than 6 months of age. Similar considerations, namely age distribution and location, were adduced by Mays (1993) as proof of infanticide in Roman Britain.

Classical authors provide ample and credible evidence for infanticide in Graeco-Roman society (cited in Brunt, 1971; Eyben, 1980–81; Pomeroy, 1984; Wiedemann, 1989; Harris, 1994). Recent archaeological discoveries attest to infanticide from one end of the Roman empire to the other, from Ashkelon in Roman Palestine (Smith & Kahila, 1992) to sites in Roman Britain (Mays, 1993).

Once accepted as a parental prerogative, official attitudes towards infanticide have changed over time. Currently illegal in most societies, the practice of infanticide is still widespread for a variety of cultural and economic reasons (Langer, 1974; Williamson, 1978; Tooley, 1983). The justification for infanticide rests in part on the assumption that new born infants are not fully human, and in part on the importance of controlling the size and the structure of the family and society at large (Tooley, 1983).

Infanticide was often preferable to abortion as a method of birth control and family planning: it allowed for sex selection and birth order to be taken into account, and it was less dangerous to the physical well-being of the mother (Eng & Smith, 1976; Stager & Wolff, 1984). In Roman society according to Cicero, malformed infants had to be destroyed, but many healthy infants were also killed. The emperors Augustus and Claudius issued edicts ordering the death of infants born to members of their family accused of adultery.

The gender of a child was often an important factor in deciding its fate. Most parents raised at least one boy as an heir or support in old age. In contrast girls, especially in patriarchal societies, were viewed as burdens, especially if their marriage was dependent on a dowry. In Roman society it was the father's decision alone that determined whether a new born baby should be permitted to live. Under some circumstances girls who could be raised as performers or prostitutes were preferentially kept (Fantham *et al.*, 1994).

The general consensus is that in both ancient and modern societies more daughters than sons were selected for infanticide (Pomeroy, 1983: 208). The most vivid and explicit reference is from a letter, dated June 17, 1 BC, written by a certain Hilarion in Alexandria to his expectant wife Alis in Oxyrhynchus. He writes "I ask and beg you to take good care of our baby son, and as soon as I receive payment I will send it up to you. If you are delivered of child [before I get home], if it is a boy keep it, if a girl discard it" (Papyrus Oxyrhynchus 744, translated in Lewis, 1985: 54). Thus, it comes as something of a surprise to find so many boy infants discarded in the sewer of Late Roman Ashkelon. Males were present at significantly higher frequencies in our subsample of 19, and there is only a very low probability (<0.001) that they were present in as few as 40% of the entire sample (confidence levels for small samples quoted in Simpson, Roe & Lewontin, 1960: 199).

Bathhouses, both public and private, proliferated throughout the Roman empire. Until the time of the emperor Claudius, men and women bathed separately. During his reign a single set of bathrooms replaced the double set as mixed bathing came in vogue, over the objections of some Romans, who were concerned that bathhouses were becoming bordellos (Ward, 1992).

Ovid, writing in the time of the emperor Augustus, offers this advice to a young woman on how to elude the guardian and have sex in the baths: "While the guardian keeps the girl's clothes without, the numerous baths hide furtive [i.e. sexual] sport" (*Ars amatoria* 3.939–40). Another author, writing in the time of Nero, describes a father who went to the baths, leaving one child at home, only to return from the baths a prospective father of two more (Ward, 1992: 134, citing Nicharchus *Anthologia Graeca* 11.243). In his epigrammatic depiction of Roman life in the 1st century AD, the poet Martial wrote: "The bathman lets you in among the tomb-haunting whores only after putting out his lantern" (*Epigrams* 3.93, translated by D. R. Shackleton Bailey in *Loeb Classical Library*, vol. 1, p. 269).

At the time when the Ashkelon bathhouse was in use, the last great Roman historian Ammianus Marcellinus (c. AD 330–395) records that when nobles, "each attended by fifty servants, have entered the vaulted rooms of a bath, they shout in threatening tones: 'Where on earth are our attendants?' If they have learned that an unknown courtesan has suddenly appeared, some woman who has been a common prostitute of the crowd of the city, some old strumpet, they all strive to be first to reach her . . . and extol her with such disgraceful flattery as the Parthians do Samiramis, the Egyptians their Cleopatras . . ." (Ammianus Marcellinus 28.4.9, translated by J. C. Rolfe in *Loeb Classical Library*, vol. 3, pp. 141–143).

There were both public and private bathhouses in Roman Ashkelon. The small bathhouse, where infants were discarded in the sewer beneath, was probably one of many private baths run for profit in this seaport. The proprietor welcomed sailors, merchants and anyone else into the bathhouse with this enticing signpost: "Enter, enjoy, and . . .".

The bathhouse was built over earlier Roman villas, including one with a room full of lamps decorated with erotic images. The bathhouse was situated in what was probably a well established part of the "red-light" district of Roman Ashkelon. The linkage of baths with prostitution has been alluded to by classical authors (cited in Dauphin, 1996) and reinforced by the architectural and epigraphic remains from Ashkelon. The presence of both male and female victims in the gutter beneath the bathhouse raises the intriguing possibility that these infants may have been the unwanted offspring of courtesans serving in the bathhouse, thus providing further supportive evidence for its use as a brothel.

At the same time this explanation may account for the predominance of male infants discarded (assuming that the limited subsample in which sex could be determined is representative of the total population of infanticide victims). Although both sexes were recruited to work as prostitutes in the bisexual world of the Romans, females were in greater demand. In the Roman empire one of the primary sources of prostitution was abandoned children who had been rescued and reared to work as prostitutes at an early age (Rousselle, 1996: 299). We can imagine that the courtesans of Ashkelon selectively kept and reared some of their illegitimate offspring (mostly females) in the profession and discarded others.

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