

u Sexing of Romano-British baby burials from the Beddingham and Bignor villas

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A simple method is described for measuring the depth of the sciatic notch in fetal ilia. In the pilot study presented, both morphological and molecular analyses were undertaken separately to determine the sexes of six Romano-British infant burials from the Roman villa at Beddingham, East Sussex and one from the villa at Bignor, West Sussex. We have attempted to establish a discriminant criterion by relating morphological findings to the sex as determined by amelogenin PCR.

Simple geometrical analysis of the sciatic notch indicated that of the six infant burials at Beddingham, three were female and three were male. Using the same criteria, the sex of the Bignor baby was determined as female. Amelogenin PCR was in agreement in four cases from Beddingham (three males and one female), but owing to poor quality of DNA, comparison was excluded in two remaining burials judged as female on morphological criteria. Similarly, owing to poor DNA preservation, PCR was negative with bone extracts prepared from the Bignor baby. If the burials at Beddingham were the result of infanticide, its victims were not exclusively female.

INTRODUCTION

The difficulties in sexing infant and fetal skeletons have long been a bane to anthropologists and palaeopathologists and a number of attempts have been made to describe functions which might discriminate between the sexes. In both palaeoepidemiology and forensic practice it would be helpful to have a simple and reliable procedure by which fetal skeletons could be sexed. The advantages to the forensic pathologist are obvious; for the palaeoepidemiologist it would be helpful to decide whether there was a differential death-rate among fetuses and neonates during different periods and if so, whether this reflected social practices, such as the supposed possible infanticide of girl babies during the Roman period as historically recorded for the rich (Scarborough 1969, 209 n.50).

It is known from experimental studies in mice and amphibians that the pelvic bones show sexual dimorphism at an extremely early age (Iguchi *et al.* 1989; Uesugi *et al.* 1992a), probably due to the effects of steroid hormones *in utero* (Uesugi *et al.* 1992b), levels of which are known to differ according to sex (Robertson *et al.* 1980). There is no reason to suppose

that human fetuses differ in this respect from animals and indeed some changes have been reported. For example, the sciatic notch is said to be shorter and deeper in male fetuses than in female (Boucher 1955; 1957; Fazekas & Kosa 1978). It has also been suggested that the antero-posterior position of the point of greatest depth of the sciatic notch is dimorphic in human fetuses (Holcomb & Konigsberg 1995).

It is now possible to ascertain the sex of a fetal skeleton by extracting and amplifying genomic DNA using an amplification procedure such as polymerase chain reaction (PCR). PCR for amelogenin, the tooth enamel protein, has been widely used in forensic medicine for determining the sex of human remains (Sullivan *et al.* 1993; Strom & Rechitsky 1998) and is increasingly finding applications in palaeopathology and bioarchaeological investigations (Stone *et al.* 1996; Faerman *et al.* 1997). Whilst this test may be available in specialized centres, it is relatively expensive to perform. Moreover, PCR relies on the survival of DNA in sufficient quantities and in a form suitable for amplification. The need for a simple discriminant procedure to sex fetuses, therefore, is still necessary.

We describe here a simple technique for

measuring the depth of the sciatic notch in fetal ilia and we have attempted to establish a discriminant criterion by relating our results to the sex as determined by PCR.

METHODS

SPECIMENS

Six fetal skeletons were made available from the site of the Roman villa at Beddingham, East Sussex and a seventh skeleton came from the 1996 season of excavation at Bignor Roman villa, West Sussex. They were examined and assigned an age based on long-bone measurements using the tables of Fazekas and Kosa (1978), and the linear regression equations of Scheuer *et al.* (1980). Their ages (in weeks gestation) are shown in Table 1.

Contexts of burials

a. The Beddingham villa, East Sussex

Excavations between 1987 and 1992 fully exposed the main villa building (*see* Fig. 1) and sampled adjacent buildings and the villa farmyard/ditched enclosure (Rudling 1998). This phase of occupation at the site may have started in the very late Iron Age or early post-conquest period and probably included the erection of a two-phase timber round structure (perhaps a house or shrine: *see* Fig. 1). The main phase of occupation at the villa, during which a masonry house was constructed, dates from the late 1st to the mid-4th centuries. The locations of the seven infant burials found at the Beddingham villa are shown on Figure 1. All were in or near the masonry house and the timber round structure referred to above.

On the basis of associated finds and stratigraphy, the earliest of the burials were probably 683, 684, 685 and 838, to the west of the timber round

structure. Burials 683, 684 and 685 were found at the same level within a deep pit (608). A complete pot dating to the 1st century (and probably pre-Flavian) was recovered from the pit at a deeper level than the infant remains. Burials 683 and 685 were flexed with the body laid on the right side. 683 was orientated (head/feet) approximately south-west/north-east and 685 approximately north-east/south-west. Burial 684, which was less intact, was orientated south/north. Burial 838, which was located within a metre of Pit 608, was badly disturbed and fragmented, and was not sexed as part of this research project. This burial was recovered from a layer of subsoil which yielded pottery dating to the 1st and 2nd centuries.

Burial 162, which was discovered in the west range of the main masonry house, was flexed, laid on its right side, and orientated east/west. The burial was inserted into a deposit containing pottery dating mainly to the 1st and 2nd centuries, but also including some late 3rd-century material. Also within the masonry building was Burial 686 which was found within a 3rd-century infill of the north wing. This burial was again flexed, but in contrast to the burials described above, had been laid on its left side. It was orientated south-east/north-west. The final infant burial at Beddingham (697) was discovered immediately to the west of the western range of the masonry house. It was flexed, laid on its right side, and orientated west/east. Dating evidence for this burial is not good, but pottery finds from the overlying deposit are mostly 1st-century.

b. Bignor villa, West Sussex

The large and luxurious villa at Bignor was discovered in 1811 and has been the subject of various, including ongoing, excavations (Rudling 1998). Despite the extensive programmes of excavation work, the site has so far yielded only one infant burial. This situation may be due to the acidity of the soil (the site is located on Upper Greensand) which is not generally favourable for the survival of small bones. The Bignor baby remains (341) were found in a pit (325) some 10 metres to the east of the ambulatory of the 4th-century courtyard villa. The burial, which was unfortunately much disturbed, is not well dated; the pottery evidence from Pit 325 consisted of only three sherds of late 1st-century pottery. A later date is possible, however, since the deposit overlying the pit yielded a mixture of 1st- to 3rd-century pottery.

Table 1. Estimated gestational age of infant burials. Values calculated from the multiple linear regression equations of Scheuer *et al.* (1980), are the best single estimates from the ICH data (for fetuses of known sex), \pm the standard error of the estimate.

Burial no.	Gestational age (wks) (after Fazekas & Kosa)	Gestational age (wks) (after Scheuer <i>et al.</i>)
162	36	37 \pm 1.9 (fem + tib)
683	36-40	41 \pm 1.9 (fem + tib)
684	36-40	42 \pm 1.7 (fem + tib)
685	36-40	40 \pm 1.7 (fem + tib)
686	36-40	39 \pm 1.7 (fem + tib)
697	36-40	39 \pm 2.22 (femur)
Bignor baby	40+	41 \pm 2.22 (femur)

Beddingham Roman Villa Baby Burials

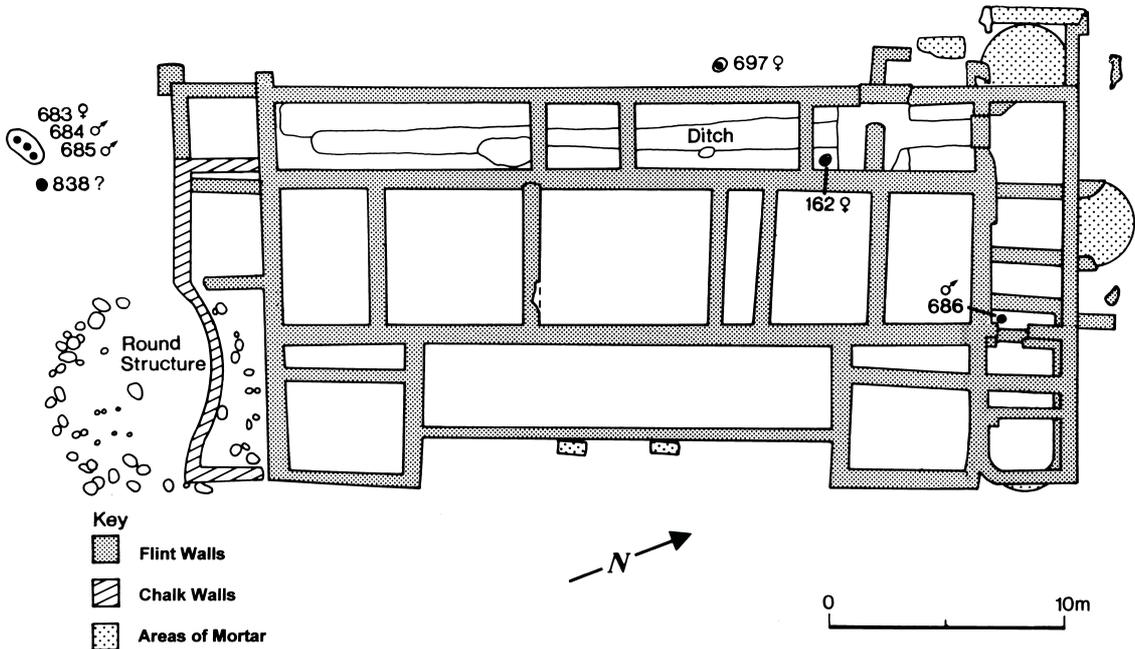


Fig. 1. Plan of burials at the Beddingham site.

Morphology of sciatic notch

Figure 2 shows the unfused bones of the innominate in early childhood, with the arrow on the ilium indicating the greater sciatic notch. The left ilium of each fetus was scanned into a personal computer and the image digitally enlarged. A line a, a' was drawn to join the two contraflexion points on the sciatic notch (as defined by Fazekas & Kosa 1978) and a tangent to the top of the curve of the sciatic notch (b, b') was drawn parallel to a, a'. A schematic plan of these points is shown in Figure 3. A perpendicular (B, C) was drawn to the tangent and this distance was measured, as was the distance A, C, where A is the point at which a, a' cuts the point of contraflexion adjacent to the articular surface of the ilium. $\tan \theta$ was calculated from BC/AC and from this the value of θ was derived. As the depth of the sciatic notch and the highest point moves forward, so values of θ will increase. Values of θ were compared with the results obtained by sexing through PCR (described below) to see if a reliable male/female cut-off point could be established. Morphological and molecular analyses were performed separately by TW and GMT respectively

and results only compared at the end of the pilot study.

Specimens for PCR

Vertebral bodies from the Beddingham infant burials and the Bignor baby were chosen as the source of bone powder. To minimize the likelihood of modern contamination through handling, the exterior 1–2 mm of the vertebral bodies were removed using sterile scalpel blades.

Two separate bone extracts were prepared from each Beddingham infant burial (Nos 162, 683, 684, 685, 686, 697 and from the Bignor baby). In addition, a third extract was prepared from the pelvises of skeletons 683, 684 and 686. The weights of bone powder ranged from 100–250 mg in each extract. In initial studies, extraction was in Tris buffered GUSCN containing EDTA as previously described (Taylor *et al.* 1996). In later experiments the NucliSens™ DNA isolation kit from Organon Teknika was assessed in parallel. Both procedures are essentially similar and are based on the method of Boom *et al.* (1990).

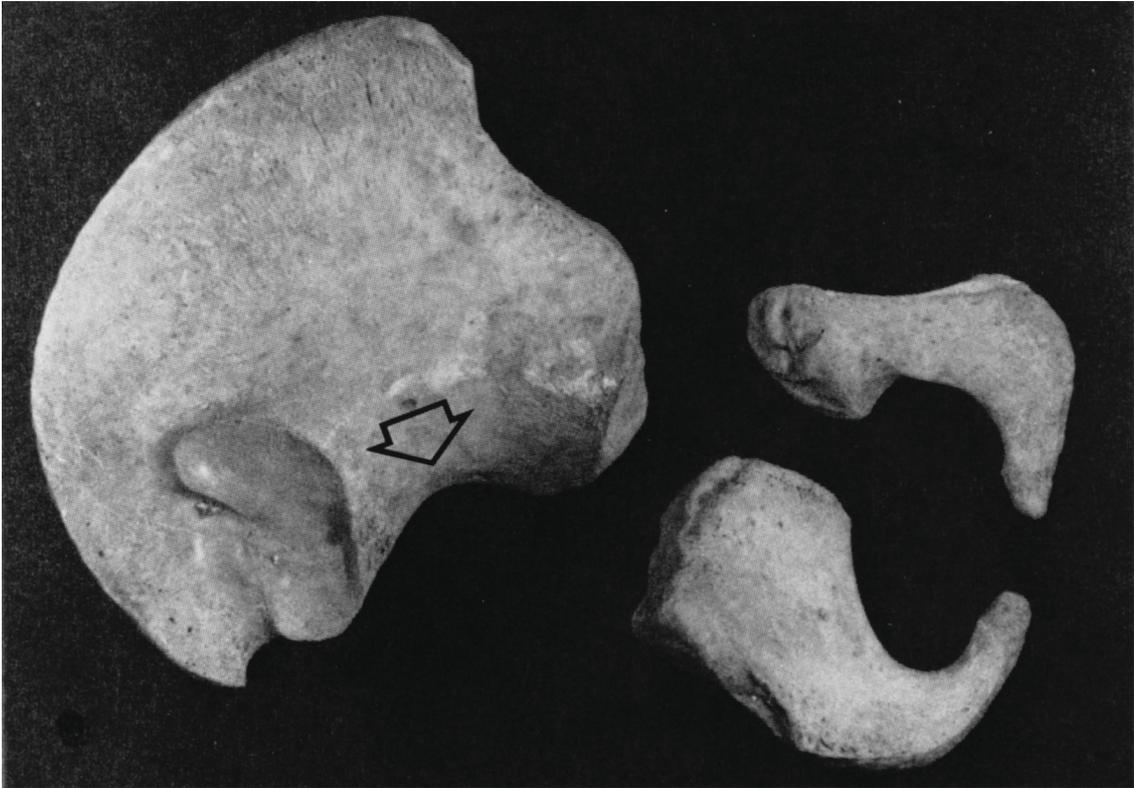


Fig. 2. Medial view of the ilium, ischium and pubis, the bones which comprise the innominate, as yet unfused in this example from early childhood. The position of the greater sciatic notch is arrowed.

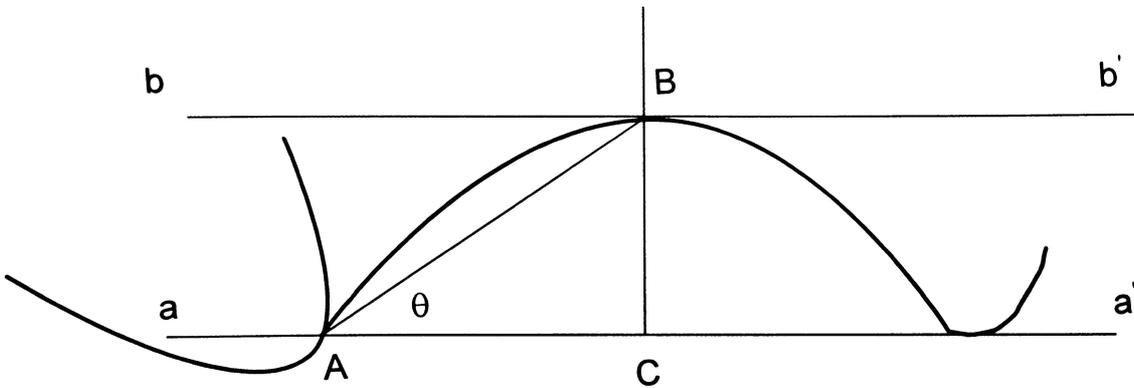


Fig. 3. Schematic plan of the fetal pelvis and sciatic notch showing derivation of the terms used to calculate the angle θ .

Principle of DNA amplification by PCR
 PCR is a technique which allows sequences of DNA present in only a few copies to be amplified *in vitro* such that the amount of amplified DNA can be visualized and identified. The requirements for PCR

are deoxynucleotides (dNTP's) a thermostable DNA polymerase enzyme, amplification buffer containing magnesium, the DNA template to be amplified and two specific oligonucleotide primers to initiate synthesis of new strands. The primers are designed

to be complementary to opposite strands of the DNA template. After heat denaturation of the template and cooling to their annealing temperature, the primers bind and initiate extension in the 5' to 3' direction, such that each primer directs synthesis towards the other. The size of the PCR product is governed by the distance along the genome between the 5' ends of the primers. Under optimized conditions of template, magnesium ion concentration and an excess of dNTP's and primers, repetitive cycles of denaturation (94°C), annealing (usually 55–68°C) and extension (72°C), will result in an exponential increase of the template.

Amelogenin PCR

Method 1

A hemi-nested PCR method was used to amplify a fragment of the genomic DNA coding for the tooth enamel protein amelogenin. Primers were designed to span a deletion in intron 1 of the Y-chromosome homologue, resulting in different sized products from the X and Y chromosomes. The sequence of the primers was:

forward (F1) 5'-

CTGATGGTTGGCCTCAAGCCTGTG-3'

second forward (F2) 5'-

TGACCAGCTTGGTTCTAWCCC-3' and

reverse (R1) 5'-CARATGAGRAAACCCAGGGTTCCA-3'.

Primers were synthesized by Genosys Biotechnologies (Europe Ltd), Cambridge, UK. In females, these primers generate a single final product of 290 bp. In males, an additional band of 105 bp is amplified.

A 'hot-start' PCR, with 35 cycles of amplification using primers F1 and R1 was performed as previously described (Taylor *et al.* 1997). Template blanks, with water in place of sample were always included.

In the second round of amplification, 1 µl of products from the first round were transferred to new tubes containing the hemi-nested primers (F2 and R1) and fresh reaction mix. Then followed a further 35 cycles of amplification (95°C for 15 s, 56°C for 40 s and 72°C for 25 s).

Method 2

In addition, the method of Sullivan *et al.* (1993) was applied to extracts from the Bignor baby. This employs a single primer pair to amplify 106 bp and 112 bp products from the X and Y amelogenin homologues respectively. After hot start, 45 cycles were run (60°C for 30 s, 72°C for 10 s and 94°C for 20 s).

All PCR was performed on a Hybaid Touchdown

sub-ambient thermal cycler (Hybaid, Teddington, Middlesex, UK).

PCR sensitivity

The detection limits of the amelogenin PCR methods were determined using partially purified human genomic DNA (105 µg/ml) as template. Serial dilutions from 10⁻¹ to 10⁻¹⁰ of this were prepared and 5 µl aliquots were used as template in the hemi-nested PCR and in a modified second round PCR.

Gel electrophoresis

Routine gel electrophoretic analysis of products was performed on either 3 per cent (PCR method 1) or 5 per cent (PCR method 2) agarose gels. The DNA bands were stained with ethidium bromide and visualized under UV illumination. Products for sequencing were subsequently run on 0.8 per cent L.M.P agarose (Gibco BRL, Life Technologies, Paisley, UK). Bands were excised from the gel with a sterile scalpel blade and purified using the NucleiClean™ DNA isolation kit (D-RAP), (Sigma/Aldrich Chemical Co., Poole, Dorset, UK).

Automated DNA sequencing

Sequencing of PCR products was accomplished using cycle sequencing with the ABI Dye Terminator Ready Reaction kit (Perkin-Elmer Applied Biosystems, Warrington, Cheshire, UK) according to the manufacturers' instructions, with subsequent analysis on an ABI 310 Genetic Analyser.

Avoidance of contamination

A number of precautions were taken to avoid contamination with modern DNA. These have previously been described (Taylor *et al.* 1996; 1997).

RESULTS

AGE OF FETUSES AND SCIATIC NOTCH MEASUREMENTS

It can be seen from Table 1 that all the fetuses were between 37 and 42 weeks gestation, which suggests that they had reached full term or had died within a week or so of birth.

The angle of θ ranged from 20°–40° (see Table 2) and the lowest values for which there was confirmation of sex by PCR (26°) was from a female; the highest values for which there was PCR confirmation (40°, 30° and 29°) were all from males. From this small series of observations it seems that values of 26° or less are likely to be female and that

Table 2. Comparison of osteology with amelogenin PCR methods for sex determination.

Burial no.	Tan θ	BAC	Sex	Amelogenin PCR
162	0.3727	$\cong 20^\circ$	Female	DNA negative
683	0.4802	$\cong 26^\circ$	Female	Female
684	0.5777	$\cong 30^\circ$	Male	Male
685	0.5516	$\cong 29^\circ$	Male	Male
686	0.8248	$\cong 40^\circ$	Male	Male
697	0.3947	$\cong 22^\circ$	Female	DNA negative
Bignor baby	0.4025	$\cong 22^\circ$	Female	DNA negative

we can separate males from females when the angle is 30° or greater.

AMELOGENIN PCR

The main findings are summarized within Figure 4. Sex, as determined by amelogenin PCR (method 1) was successful with samples 683, 684 and 686 although in the case of the latter, amplification was sample dependent, i.e. DNA was amplified from two vertebral bodies but not with the sample taken from the pelvis. Otherwise, duplicate analyses were performed on each extract and yielded consistent results. Burials 162 and 697 were negative by PCR (method 1) and this is considered to be due to poor DNA survival. The same was true of the Bignor baby burial from West Sussex, which in addition was negative by a second amelogenin PCR which amplifies smaller product sizes of 106 and 112 bp. Standard methods of overcoming PCR inhibition, such as addition of bovine serum albumin (BSA) and extra units of DNA polymerase to the PCR master-mix of these samples were without effect. We therefore conclude that failure of PCR was due to lack of sufficient template in the skeletons, rather than co-purification of PCR inhibitors. A sixth sample from Beddingham, burial 685, produced only the Y-chromosome band with hemi-nested PCR (105 bp, see Fig. 4, lane 6) and this implies that DNA fragments in the extract were at least 256 bp in length but less than 436 bp, the sizes of the first-round Y- and X-chromosome templates respectively. As this single band pattern was a consistent finding in two extracts, failure to amplify the female product is unlikely to be due to allele 'drop-out'.

SENSITIVITY

Using hemi-nested PCR, the lowest dilution of the stock in which products were detected in $5 \mu\text{l}$ was at 10^{-5} , corresponding to 5.25 pg.

DNA SEQUENCING

The single band from sample 685 (see Fig. 4) was

sequenced to confirm its identity as the Y-chromosome specific product of 105 bp. With the forward primer, 95 per cent homology was obtained over 64 bp downstream from the primer. Sequencing with the reverse primer was unsuccessful. This is thought to be due to insufficient DNA template.

DISCUSSION

The identification of the sex of neonates and larger infants from burials at archaeological sites remains problematical. Advances in molecular biology have resulted in methods for sex identification based on polymorphisms on the sex chromosomes. Variations in the amelogenin gene, present on both X and Y chromosomes, have formed the basis for several molecular methods. We describe here a PCR test for amelogenin which results in products from the X and Y chromosomes which may be easily distinguished by routine agarose gel electrophoresis. Some caveats remain. Variable DNA survival at sites is to be expected; PCR is expensive and not always available for post-excavation analysis. Therefore there remains a need for an easily-applied osteological procedure for sex identification of infants.

Whilst studying the infant remains from Beddingham, we have taken the opportunity to compare an osteological method of sexing human infant remains, based on morphology of the sciatic notch, with our PCR method for amplification of DNA coding for the tooth enamel protein amelogenin. The morphological method described is relatively simple, certainly less complicated than that described by Holcomb and Konigsberg (1995), but it incorporates the observation from their study that the maximum depth of the sciatic notch varies in its a/p position according to sex, being located more anteriorly in males. Although this was found to be the most dimorphic feature of the fetal pelvis, these authors considered that the amount of overlap between males and females was so great that the sciatic notch could not be used as a reliable sex indicator. However, the angle of θ (Fig. 3) depends for its value on both the depth of CB and the position of the point B; it will increase as CB increases and as B moves anteriorly.

The results of this small pilot study suggest that when values for θ are 26° or below, then the pelvis is female and that when θ is greater than 30° , the pelvis is male. We consider that some overlap in values of θ is bound to occur. We have not succeeded

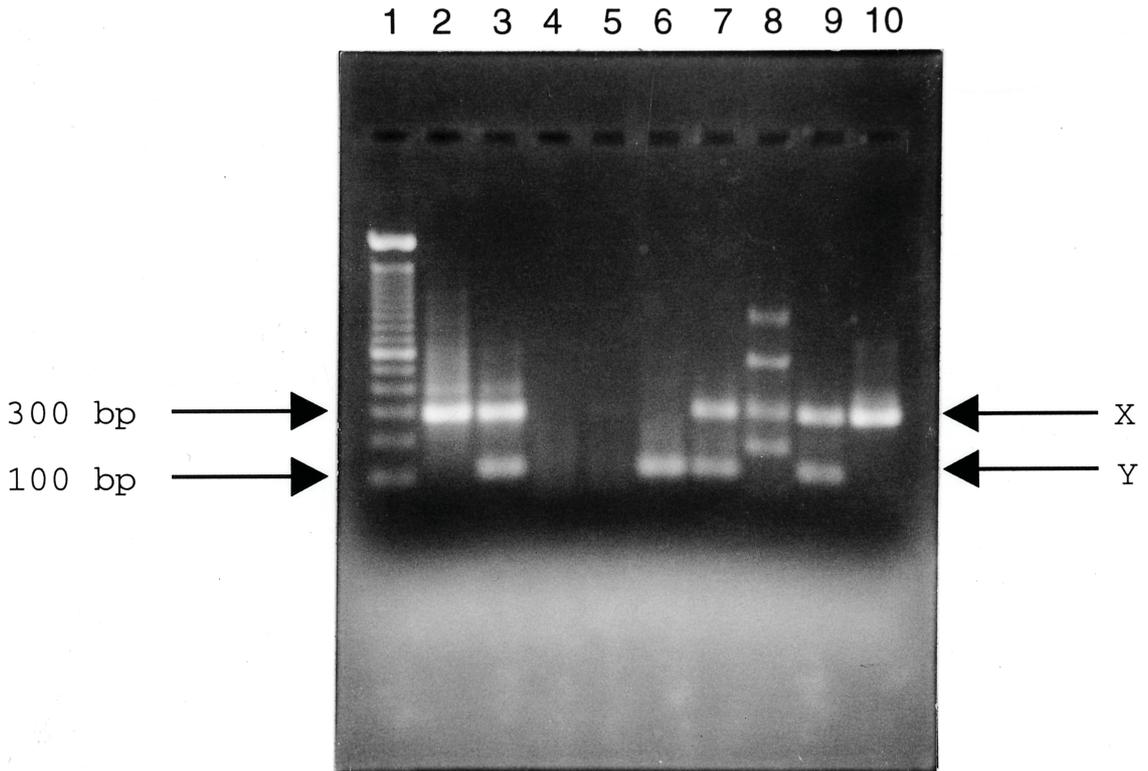


Fig. 4. Amelogenin PCR, method 1. Electrophoretic analysis of products on 3 per cent agarose gel. Lane 1: 100 bp DNA size markers. Lane 2: Female DNA standard. Lane 3: Male DNA standard. Lane 4: Template (water) blank. Lane 5: Bignor Baby extract. Lane 6: Burial 685. Lane 7: Burial 686, vertebral body extract. Lane 8: Burial 686, pelvic extract. Lane 9: Burial 684, pelvic extract. Lane 10: Burial 683, pelvic extract.

in our primary aim of being able to provide a discriminatory value for θ (which will presumably lie above 30°) but we consider the method is sufficiently promising to justify further investigation. We would anticipate that discriminant values of θ may be population dependent and would need to be determined for each group studied.

The sample size studied was too small ($n = 7$), to permit any statistical comparison of the morphological and PCR methods. However, agreement was found in four of the samples studied which were from the Beddingham site. There are therefore reasonable grounds for attributing sex to burials 683, 684, 685 and 686 (Table 2).

DNA survival was variable at the Beddingham villa, but was satisfactory in infants 683, 684 and 686 which were recovered from two different contexts (*see above*). We attribute amplification failure of the X-chromosome fragment in burial 685

(Fig. 4) to the fact that DNA template fragments were more degraded in this sample. Indeed, the design of primers and the expected product sizes has permitted some conclusions to be inferred concerning the length of surviving DNA fragments in excavated bones. Poor-quality DNA from burials 697 and 162 prevented PCR comparison with the morphological method. The same was true of the Bignor baby remains where even a second PCR method, amplifying shorter DNA fragments, was negative. The acid soil conditions at Bignor might account for the loss of DNA from this specimen as DNA preservation is improved under neutral or slightly alkaline conditions (Burger *et al.* 1999).

The fetuses would have been viable at 38–40 weeks, the ages attributed to the burials at the villas. Therefore the question arises as to whether the infant burials at Beddingham and Bignor represent stillbirths, deaths due to disease in the first few days

of life or infanticide.

Certainly there are literary sources which refer to infanticide in the Roman Empire and the practice was probably widespread (Harris 1994; Scarborough 1969; Wiedemann 1989).

It is important to remember that infant remains at Roman sites might reflect a combination of all three possibilities. It is certainly true that one cannot know precisely the cause of death in a full-term infant; it may be due to birth trauma or to congenital defects — although fatal defects are extremely rare in full-term infants.

In attempting to unravel this problem, Mays (1993) has studied the gestational ages of a total of 164 infant burials on later Romano-British sites. These included villas, settlements and cemeteries. By comparing the distribution of their gestational ages with a) modern live births, b) modern stillbirths and c) modern live births dying within one week, he was able to show that the age distributions from the Roman sites, like the modern live births, were centred around full term. This argues in favour of infanticide, as this is usually carried out soon after birth. In contrast, modern data of stillbirths and infants dying within one week of birth were skewed towards the lower gestational ages. There are too few infant remains from Beddingham to subject to this analysis. However, the clustering of gestational ages around full term is characteristic of the findings

at other Roman sites and infanticide cannot be excluded.

Female infanticide has occasionally been suggested to account for the higher proportion of adult males in Romano-British cemeteries, i.e. 1.46:1 (Mays 1995). If the burials at Beddingham do represent infanticide, then the presence of three male infants exclude the practice having been directed exclusively at new-born females. A DNA study of the Roman Ashkelon infanticide victims (Faerman *et al.* 1997) has also revealed a high proportion of males, and although DNA analysis was limited to 19 cases, only 5 of these were female. In view of the earlier DNA findings from Ashkelon and now Beddingham, the need for a larger combined morphological and molecular study of the Romano-British material is indicated.

It would seem that the burial of the remains of any older infant fatalities occurred away from the settlement site. For further discussions of infant burials on Romano-British occupation sites see Collis (1977); Scott (1990; 1991; 1992); Mays (1993) and Harris (1994).

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