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Verifying archaeological hypotheses: Investigations on origin and genealogical lineages of a privileged society in Upper Bavaria from Imperial Roman times (Erding, Kletthamer Feld)

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Abstract
During the years 2005 and 2006 approximately 2000 archaeological finds ranging from the Neolithic Period to Late Antiquity were found on the Kletthamer Feld (Erding, Upper Bavaria). Out of this context a burial site was examined comprising 13 individuals, some of them rich in precious grave goods. The inhumations were dated to the second half of the 4th to the first half of the 5th century – a time of upheavals in relation to the demographic structure of the former Roman province Raetia (today southern Bavaria).

The high proportion of male individuals within the skeletal population as well as the finding of a Roman fibula, which is seen as part of Roman military clothing, led to distinct hypotheses which we have attempted to support in this study. The hypothesis that the skeletal remains reflect a founder population from a Germanic region north of the Danube River could be rejected on the basis of stable isotope analyses. The theory of a buried family clan had to be dismissed as well, or rather, be extended to the scenario of several families being buried there with their servants. The results obtained fit the third presumption best, namely that the buried individuals were the members of a military unit interred with their families.

Keywords
late roman imperial period; stable isotope analyses; kinship analyses

Introduction

In 2005 and 2006 a small yet remarkable burial site was found in the east of Erding (Kletthamer Feld, Upper Bavaria). It consisted of 13 inhumations and was dated to the second half of the 4th to the first half of the 5th century.

The cemetery can be assigned to the Late Imperial Roman Period and thus falls into a rarely investigated transition period between Roman Times and Migration Period – a time of social upheavals in the former Roman province Raetia (today southern Bavaria):

After the Roman occupation of the Alpine foothills 15 BC, today’s Bavaria underwent several years of intense Romanization, leading to considerable affluence in many places (Fehr, 2010). From the middle of the third century AD onward, continuous attacks by Germanic tribes, who repeatedly forced their way into the Roman territory, finally resulted in the settlement of non-Roman inhabitants in Raetia. Due to the ongoing attacks, the recruitment of Germanic mercenaries for the Roman army had already become more and more common decades before, and hence the military force in the west of the empire in the 4th century was largely composed of barbarians (Steidl, 2006). The dethronement of the last Roman emperor by the barbaric military commander Odoaker in the year 476 heralded the end of the Roman rule north of the Alps. It seems that in the course of the general economic decline during the years that followed, supraregional trading networks gradually collapsed, the population as well as the army size decreased and Roman soldiers ceased to receive salaries towards the end of the 5th century.
Furthermore, an increase in the influx of Germanic immigrants from regions north of the Danube River set in (Fehr, 2010).

The cemetery was located within a large complex of prehistoric and Roman archaeological findings, though remnants of settlement features specifically associated with the burials could not be found. A remarkable discovery was that of abundant funerary goods in certain graves which can be considered as unique for rural Raetia at that time: three adult to mature women were buried with gold jewellery and several glass vessels, indicating wealth or a superior standard of living. The male burials contained no grave goods, except for one. The individual in question was interred with a fibula (cross-bow brooch) which is considered to be an element of Roman military clothing. The fact that grave goods were found at all suggests that the buried people were of Germanic descent. Furthermore, the ratio of sexes at the burial site reveals a striking dominance of male individuals (c.f. Table 1). Moreover, only one child could be identified.

These findings make it necessary to answer one question before further interpretations concerning the way of life of the people interred at these times can be considered: What kind of society lies buried here?

The archaeological data, the historic circumstances and the morphological results suggest several hypotheses that should be considered:

1. The age and sex distribution could be indicative of a founder population (far more males than females, Staskiewicz, unpublished report of the morphological examination) that, based on the historical background, probably consisted of Germanics from north of the Danube River

2. The deceased might be members of a single small-sized but well-situated family that lived at an estate located near the burial place that has not yet been discovered (Biermeier and Pietsch, 2006)

3. The buried men might have been members of a Roman military unit – perhaps road guards who lived there with their families

Table 1 | Morphological results and results of molecular sex typing by amplification of the X-Y homologous gene amelogenin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age at death*</th>
<th>Sex morphological</th>
<th>Sex genetical</th>
</tr>
</thead>
<tbody>
<tr>
<td>1662</td>
<td>m</td>
<td>male</td>
<td>male</td>
</tr>
<tr>
<td>1663</td>
<td>a</td>
<td>female</td>
<td>female</td>
</tr>
<tr>
<td>1664</td>
<td>m</td>
<td>male</td>
<td>male</td>
</tr>
<tr>
<td>1665</td>
<td>j – a</td>
<td>male</td>
<td>male</td>
</tr>
<tr>
<td>1699</td>
<td>a</td>
<td>male</td>
<td>male</td>
</tr>
<tr>
<td>1700</td>
<td>m – s</td>
<td>female</td>
<td>female</td>
</tr>
<tr>
<td>1702</td>
<td>a</td>
<td>female</td>
<td>/</td>
</tr>
<tr>
<td>1703</td>
<td>a</td>
<td>male?</td>
<td>male</td>
</tr>
<tr>
<td>1704</td>
<td>a – m</td>
<td>male?</td>
<td>male</td>
</tr>
<tr>
<td>1717</td>
<td>m</td>
<td>male</td>
<td>male</td>
</tr>
<tr>
<td>1719</td>
<td>m – s</td>
<td>male</td>
<td>male</td>
</tr>
<tr>
<td>1720</td>
<td>inf I</td>
<td>male?</td>
<td>/</td>
</tr>
<tr>
<td>1721</td>
<td>a – m</td>
<td>female?</td>
<td>female</td>
</tr>
</tbody>
</table>

* inf I = 1–6y, j = 13–20y, a = 20–40y, m = 40–60y, s = 60+
In this case it is possible to verify two of the theories through archaeometric analyses according to the following assumptions:

- If this was a founder population, the majority of the individuals should have spent their childhood at a location other than the area of the burial site. This can be validated by stable strontium (as geological marker) and oxygen (as ecological marker) isotope analysis, given sufficient geological and ecological differences between the place of origin and the place of destination. Differences in diet detected through stable isotope analysis of carbon and nitrogen can also be relevant for the identification of nonlocal individuals (c.f. Hakenbeck et al., 2010)

- If a single family was buried at the site, there should be a detectable genetic relationship among the individuals. The reconstruction of maternal and paternal lineages can be achieved by DNA analyses.

The third hypothesis cannot be verified by archaeometric methods. However, data acquired using these scientific methods can be evaluated in respect of the conclusiveness regarding the hypothesis in order to ascertain whether these are the remains of a small military unit buried with their family members. If so, one could expect an outcome in which the group is comprised mainly of unrelated male individuals.

Material and methods

The Late Roman cemetery Kletthamer Feld is located in Erding (Upper Bavaria, Germany) and was excavated in 2006 by Stefan Biermeier and Axel Kowalski.

Osteological analysis of the thirteen individuals was undertaken by Dr. Anja Staskiewicz. The state of preservation of bones varied widely; as a result, age at death and sex could therefore not be definitively determined in every case, an overview is given in Table 1.

Animal bones were found in some of the graves, Dr. Anja Staskiewicz performed species identification. To provide values for comparison with the human samples, stable isotope analysis of animal bone collagen and apatite was performed. Three cattle and five chicken bones were examined.

Stable isotope analyses

For the purpose of stable isotope analyses of light elements (C, N and O), human bone samples were taken from long bones (0.5 g) where possible, using a diamond-edged cutting wheel. Spongiosa was removed. The animal bones analyzed were heavily fragmented and therefore used in total.

The first molar was preferably used, whenever possible, for stable strontium isotope analyses. In cases where the first molar was unavailable, the second molar was taken. No tooth material was available in the case of Individuals 1702 and 1721, so they had to be excluded from the strontium isotope investigation.

To extract collagen, 1M HCl was added to bone powder for 20 min on a shaker for demineralization. Samples were washed, then incubated overnight, shaking in 0.125M NaOH to precipitate humic impurities. For gelatinization, samples were left in 0.001M HCl for 10–17 hours in a 90°C water bath. The gelatine was filtrated and lyophilized prior to mass spectrometry.

Carbon and nitrogen isotope analyses of “organic materials” were performed with an elemental analyser (Carlo-Erba1110) connected online to a ThermoFinnigan Delta Plus mass spectrometer. All car-
bon isotope values are reported in the conventional δ notation in per mil relative to V-PDB (Vienna-PDB). Nitrogen isotope ratios are reported in ‰ relative to atmospheric N₂ (AIR). Accuracy and reproducibility of the analyses was checked by replicate analyses of international standards (USGS 40). Reproducibility was better than ± 0.10‰ for carbon and ± 0.04‰ for nitrogen (10).

To extract the structural carbonate, bone powder was immersed in 4 % NaOCl for at least 48 hours; the solution was changed daily. All organic substances are solubilized when gas bubbles are no longer generated. Samples were incubated in 5 ml 1M calcium acetate buffer (pH 4.75) for half a day under constant motion, removing adsorbed carbonate. After being washed in distilled water, the samples were lyophilized prior to mass spectrometry.

Carbonate powders were reacted with 100 % phosphoric acid at 70°C using a Gasbench II connected to a ThermoFinnigan Five Plus mass spectrometer. All values are reported in per mil relative to V-PDB by a δ¹⁸O value of –2.20‰ to NBS19. Reproducibility was checked by replicate analysis of laboratory standards and is better than ± 0.04‰ (10).

For strontium isotopic analyses, tooth enamel was separated from dentine and then cleaned in an ultrasonic bath in concentrated formic acid for 5 min twice (acid changed) to remove surface contamination. Samples were ashed at 500°C for 12 hours. After ashing 15 to 35 mg of enamel was dissolved in concentrated HNO₃ on a heating plate at 100°C. Following vaporization of the concentrated acid, the samples were dissolved in 6N HNO₃ for separation in cation exchange columns (Sr resin, Eichrom). This step separates rubidium from strontium prior to mass spectrometry.

Samples were loaded on Wolfram single filaments. Isotope analyses of Sr were performed on a thermal ionisation mass spectrometer (TIMS, MAT 261.8, Thermo-Finnigan). Isotope mass fractionation during analysis was corrected by referencing to an invariant ⁸⁸Sr/⁸⁶Sr value of 8.37521. The achieved precision of strontium isotope measurements normally is <0.003 % (2m). A combined uncertainty (precision + accuracy) for ⁸⁷Sr/⁸⁶Sr is <0.005 %.

DNA analyses

Teeth with intact roots were preferred for DNA analyses. In the cases of individuals 1702 and 1721 no tooth material was available, so samples were taken from long bones (humerus and tibia).

The necessary precautions were observed in all preparatory steps for DNA analysis in order to avoid any form of contamination:

All pre-PCR steps were performed in a clean laboratory dedicated solely to ancient DNA, equipped with UV light in the ceiling and an UV Air Flow Cleaner (Kisker-Biotech). Full body overalls with hoods, single-use disposable gloves, hair and face masks were used. All chemicals and reagents were of analytical grade or the highest purity available. PCR tubes, reaction tubes and pipette tips were free of human DNA, as guaranteed by the manufacturer (Peqlab, Eppendorf). All pretreatment steps were performed under a Clean Air Bench and all PCR reactions were performed in an Ultraviolet Sterilizing PCR Workstation (Peqlab) inside the clean laboratory. The laboratory devices and work spaces were cleaned before and after every work-step by UV radiation and by wiping the surface with bleach.

The bone and tooth samples were cleaned mechanically with a paper cloth soaked in 1 % NaOCl solution. The tooth samples were then bathed in a 0.4 % NaOCl solution followed by subsequent washing steps with ultrapure water. After drying for about 2 days the samples were UV irradiated at 254nm for 15 min on each side shortly before the homogenisation by mortar and swing mill (Retsch).
The DNA was extracted using a modified version of the protocol of Yang and colleagues (1998) (Wiechmann and Grupe, 2005).

Mitochondrial DNA was amplified and sequenced several times from the hypervariable region 1 (HVR 1) of the mitochondrial control region using four overlapping segments (c.f. Table 2). The 25 μl PCR reaction (30–35 cycles) using the Platinum® taq high fidelity polymerase was performed as previously described in Rudbeck and colleagues (2005). PCR was followed by electrophoretic separation in an agarose gel stained with ethidium bromide. The products were extracted from the gel using the NucleoSpin® Extract II kit (Macherey-Nagel). Cycle sequencing was done with BigDye Terminator chemistry (Applied Biosystems) with either the forward or reserve primer used for amplification. Sequences were aligned against the revised Cambridge Reference Sequence (rCRS, Andrews et al., 1999) using Codon Code Aligner Software. Presumable assignments of haplogroups were implemented based on HVR I sequences using an online mitochondrial phylogenetic tree as well as a mitochondrial motif database (http://www.phylotree.org and http://mtmanager.yonsei.ac.kr/) and the corresponding literature (e.g. Alvarez-Iglesias et al., 2009; Grignani et al., 2009; Palanichamy, 2004; Richards et al., 2000; Achilli 2004, 2005). The frequencies were estimated applying the EMPOP Database (http://empop.org), developed by the Institute of Legal Medicine (GMI), Innsbruck Medical University and the Institute of Mathematics, University of Innsbruck.

To confirm haplogroups and to verify the obtained sequence motif, a single primer extension assay developed by Nelson et al. (2007) was carried out. This assay enables the determination of twelve mtDNA haplogroup specific polymorphisms (for primers, protocol and details c.f. Nelson et al., 2007).

The used SNP multiplex provides a determination of mtDNA haplogroups A, B, C, D, E, F, G, H, L1/L2, L3, M and N by typing 12 SNPs (8272–8280 del, 13263, 1719, 5178, 663, 10398, 10400, 3594, 7028, 12406, 4833, 7600).

To check on chromosomal DNA preservation and for molecular sex typing, a part of the X–Y homologous amelogenin gene was amplified using the primers published by Mannucci and colleagues (1994) and separated by polyacrylamide gel electrophoresis (c.f. protocol Wiechmann and Grupe, 2005). Furthermore, comparing the results of the molecular sex typing with the morphological sex typing can give evidence for the authenticity of DNA amplification (Meyer et al., 2000).

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’→3’)</th>
<th>Fragment</th>
<th>Length of amplification product</th>
<th>Annealing-temperature</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>L16055</td>
<td>GAAGCAGATTGTTGACAC</td>
<td>1A</td>
<td>123bp</td>
<td>56°C</td>
<td>Rudbeck et al. 2005</td>
</tr>
<tr>
<td>H16139</td>
<td>TACTACAGGTTGTAAGAT</td>
<td>1B</td>
<td>126bp</td>
<td>56°C</td>
<td>Gabriel et al. 2001</td>
</tr>
<tr>
<td>L16131</td>
<td>CACCATGAATATTGTACGTT</td>
<td>2A</td>
<td>133bp</td>
<td>46°C</td>
<td>Gabriel et al. 2001</td>
</tr>
<tr>
<td>H16218</td>
<td>TGTGTGATAGTTGAGGGTTG</td>
<td>2B</td>
<td>143bp</td>
<td>52°C</td>
<td>Gabriel et al. 2001</td>
</tr>
</tbody>
</table>
Table 3 | Stable light isotope and strontium isotope data of human samples. 1699 was excluded due to poor preservation, 1717 was used up in mass spectrometry. 1702 and 1721 had no teeth for strontium analysis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Collagen δ¹³C vs. V-PDB</th>
<th>Collagen δ¹⁵N vs. AIR</th>
<th>Collagen C/N molar</th>
<th>Carbonate δ¹⁸O vs. V-PDB</th>
<th>Strontium Tooth</th>
<th>Ratio ⁸⁷Sr/⁸⁶Sr</th>
</tr>
</thead>
<tbody>
<tr>
<td>EKF 1662</td>
<td>–19.9</td>
<td>10.0</td>
<td>3.2</td>
<td>37.7</td>
<td>13.8</td>
<td>–8.4</td>
</tr>
<tr>
<td>EKF 1663</td>
<td>–19.5</td>
<td>9.1</td>
<td>3.1</td>
<td>35.1</td>
<td>13.1</td>
<td>–9.8</td>
</tr>
<tr>
<td>EKF 1664</td>
<td>–19.8</td>
<td>9.1</td>
<td>3.2</td>
<td>38.8</td>
<td>14.1</td>
<td>–8.7</td>
</tr>
<tr>
<td>EKF 1665</td>
<td>–19.7</td>
<td>9.0</td>
<td>3.1</td>
<td>32.6</td>
<td>12.3</td>
<td>–8.9</td>
</tr>
<tr>
<td>EKF 1699</td>
<td>–20.0</td>
<td>11.2</td>
<td>2.6</td>
<td>33.6</td>
<td>15.0</td>
<td>–8.6</td>
</tr>
<tr>
<td>EFK 1700</td>
<td>–19.5</td>
<td>9.4</td>
<td>3.1</td>
<td>39.6</td>
<td>15.0</td>
<td>–8.1</td>
</tr>
<tr>
<td>EFK 1702</td>
<td>–19.7</td>
<td>10.0</td>
<td>2.9</td>
<td>39.5</td>
<td>15.7</td>
<td>–9.9</td>
</tr>
<tr>
<td>EFK 1703</td>
<td>–19.5</td>
<td>8.9</td>
<td>3.2</td>
<td>40.4</td>
<td>14.9</td>
<td>–8.3</td>
</tr>
<tr>
<td>EFK 1704</td>
<td>–20.0</td>
<td>9.4</td>
<td>3.2</td>
<td>38.8</td>
<td>14.1</td>
<td>–9.1</td>
</tr>
<tr>
<td>EKF 1717</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–9.1</td>
</tr>
<tr>
<td>EKF 1719</td>
<td>–19.4</td>
<td>9.7</td>
<td>3.2</td>
<td>39.0</td>
<td>14.1</td>
<td>–8.8</td>
</tr>
<tr>
<td>EKF 1720</td>
<td>–19.5</td>
<td>12.0</td>
<td>3.1</td>
<td>38.2</td>
<td>14.2</td>
<td>–8.9</td>
</tr>
<tr>
<td>EKF 1721</td>
<td>–17.0</td>
<td>10.4</td>
<td>3.1</td>
<td>32.5</td>
<td>12.4</td>
<td>–7.7</td>
</tr>
<tr>
<td>Mean</td>
<td>–19.5</td>
<td>9.8</td>
<td>3.1</td>
<td>37.1</td>
<td>14.0</td>
<td>–8.8</td>
</tr>
</tbody>
</table>

Fig. 1 | Carbon and nitrogen values of human and animal samples.
When a male sample showed positive amelogenin results, Y-chromosomal STRs were amplified using the Mentype® Y-MHQS PCR Amplification Kit (Biotype), according to the manufacturer’s instruction, with 35 cycles each. This amplified the nine Y-chromosomal STR loci of the minimal haplotype standard.

Further steps taken to ensure the authentication of the results were:

- Negative controls accompanied every extraction and PCR.
- To exclude the possibility that a member of the laboratory staff or the anthropologist who did the cleaning and morphological investigation of the skeletons was the source of the amplified DNA, all aDNA sequences obtained were compared with the HVR 1 sequences and the Y-STR profile of the individuals in question.
- Only sequences of the HVR 1 region which could be reproduced at least three times from two different teeth were taken into account.
- Chromosomal Y-STR analyses were also carried out at least three times per individual to avoid erroneous allele determination.

Results and Discussion

Reconstruction of diet and provenance by stable isotope analyses

Bone collagen provides the basis for reconstructing dietary patterns of ancient populations. It mirrors the isotopic signature of food and liquids consumed during the last years of an individual’s life. By measuring the ratios of stable carbon and nitrogen isotopes ($\delta^{13}$C, $\delta^{15}$N) the origin of the protein portion of a diet can be ascertained (Ambrose, 1990; Katzenberg, 2000; Schoeninger and DeNiro, 1984). It should be borne in mind that in the course of degradation processes during the inhumation period losses of amino acids, e.g., can occur, leading to alterations of the original isotopic signal (e.g. Ambrose, 1990). For this reason, it was necessary to check the state of collagen preservation first, as the integrity of collagen is essential for the validity of the isotopic data.

All samples yielded enough collagen for mass spectrometry and fulfilled quality criteria (>0.5 w%) according to van Klinken (1999) and Harbeck and Grupe (2009). One specimen (EKF 1699) had to be excluded from further interpretations as the C/N molar ratio was below the accepted range of 2.8 to 4.0 (Harbeck and Grupe, 2009). Human isotopic ratios for carbon and nitrogen are listed in Table 3; data sets for animals are in Table 4.

Nitrogen in bone collagen is exclusively related to dietary protein. Consumers on higher trophic levels exhibit more positive $\delta^{15}$N values (Ambrose, 1993; DeNiro and Epstein, 1981; Schoeninger and DeNiro, 1984). This pronounced trophic level effect is caused by the enrichment in $^{15}$N according to species-specific metabolic pathways in steps of 3–5‰ (Minagwa and Wada, 1984; Schoeninger and DeNiro, 1984).

The adults show $\delta^{15}$N values between 8.9‰ and 10.4‰, indicating high quality, meat-based nutrition. The highest values might even suggest greater consumption of milk or egg-based products, as $\delta^{15}$N exceeding 10‰ point to food sources richer in protein than meat alone, similar to the carnivore effect in breast-fed children (Katzenberg, 2000; Richards et al., 2002). Individual 1720 represents the only child (2–3a) found in the complex. It shows the highest $\delta^{15}$N value (12.01‰), which is in accordance with signatures found in infants that are still being fed on breast milk by their mothers (for weaning effects c.f.: Katzenberg, 2000; Richards et al., 2002).
Table 4 | Stable light isotopic data of animal samples. EKF 1706 / 482–4 provided insufficient material for both apatite and collagen extraction

<table>
<thead>
<tr>
<th>Animals</th>
<th>Collagen</th>
<th>Carbonate</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ¹³C vs. V-PDB</td>
<td>δ¹³N vs. AIR</td>
<td>C/N molar</td>
</tr>
<tr>
<td>EKF 1662 / 459–2</td>
<td>−21.7</td>
<td>6.3</td>
<td>3.3</td>
</tr>
<tr>
<td>EKF 1663 / 461</td>
<td>−20.6</td>
<td>8.2</td>
<td>3.2</td>
</tr>
<tr>
<td>EKF 1663 / 462–1</td>
<td>−21.0</td>
<td>8.4</td>
<td>3.1</td>
</tr>
<tr>
<td>EKF 1663 / 462–2</td>
<td>−20.1</td>
<td>7.9</td>
<td>3.2</td>
</tr>
<tr>
<td>EKF 1664 / 465</td>
<td>−21.3</td>
<td>6.4</td>
<td>3.2</td>
</tr>
<tr>
<td>EKF 1706 / 482–2</td>
<td>−20.6</td>
<td>8.2</td>
<td>3.1</td>
</tr>
<tr>
<td>EKF 1706 / 482–3</td>
<td>−20.5</td>
<td>8.2</td>
<td>3.2</td>
</tr>
<tr>
<td>EKF 1706 / 482–4</td>
<td>−20.3</td>
<td>8.4</td>
<td>3.2</td>
</tr>
<tr>
<td>mean</td>
<td>−20.8</td>
<td>7.8</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Fig. 2 | Oxygen isotopic values of human and animal samples. The range of oxygen isotopic values for the animal samples is highlighted in grey; these have been assumed to reflect local signatures. Most humans fall within this range, excepting three individuals.
The δ¹³C of the consumer’s bone collagen reflects mostly the protein part of its diet (Ambrose, 1990). In terrestrial environments, major fractionations occur during plant photosynthesis. C₃ plants (Calvin cycle), for example, discriminate against the heavy isotope ¹³C to a significantly greater degree than do C₄ plants (Hatch-Slack cycle). The average δ¹³C value of C₃ plants is −26‰ but the actual range is rather large, due to differences in temperature and precipitation. C₄ plants are not able to thrive in dense forests, preferring warm and more arid environments. Their δ¹³C values are less variable and average about −12‰.

Apart from EKF 1721 (δ¹³C = −16.96‰), the rather narrow range of δ¹³C values varies between −19.97‰ and −19.36‰ (c.f. Table 3, Fig. 1). The cattle show an average value of −21.3‰ (Table 4, Fig. 1). They reflect the signature of typical Central European terrestrial plant consumption (Ambrose 1993).

Unlike the other individuals, EKF 1721 must have had a C₄ plant containing diet, based on the δ¹³C values. The fact that C₄ plants did not grow in Central Europe at that time means that a C₄ plant signature can be expected in bones belonging to an “immigrant” to the site (Lösch, 2006).

Hakenbeck et al. (2010) analysed several samples from different early medieval graveyards in Bavaria. Among these were also ten of the analysed individuals of Erding, Kletthamer Feld (1662, 1663, 1664, 1665, 1700, 1702, 1703, 1719, 1720, 1721). Their results were consistent with those in our study, varying only in insignificant ranges (δ¹³C ±0.42‰; δ¹⁵N ±0.68‰). These differences might be due to a slight difference in the extraction method (0.5M HCl instead of 1M HCl, no NaOH or gelatinization in 0.005M HCl).

The correlation between temperature and δ¹⁸O in water is well known, and δ¹⁸O has frequently proved to be a valid palaeothermometer in the past (Aitken, 1990). Since the δ¹⁸O in meteoric water varies with temperature, latitude, altitude and distance from the coast (Fry, 2006), δ¹⁸O in bone can serve as a valuable tool to establish the ecological origins of immigrants to the site. However, as the structural carbonate of bone reflects the isotopic signature of liquids consumed during the last years of an individual’s life, it only allows the detection of recent migration movements.

Human and animal isotopic ratios for oxygen are listed in Table 3 and 4. Individual 1702 and 1663 show the most negative δ¹⁸O values among the group (c.f. Table 3, Fig. 2). They seem to have used a drinking water source different than that used by the rest. This might be evidence of migration from a climatically different area at a higher altitude and/or cooler temperatures.

The most positive δ¹⁸O value is shown by individual EKF 1721. EKF 1721 also displayed “conspicuous” carbon values, pointing to a possible Mediterranean origin. More positive oxygen isotopic data support this assumption, as they reflect a water source from a warmer and drier area and a possible marine influence (Fry, 2006). Unfortunately, there were no teeth preserved for strontium isotope analysis to prove this supposition.

Strontium isotopes are geochemical markers used to allocate archaeological finds to a certain geological region and to trace mobility during an individual’s life time. Stable strontium isotope ratios of bones and teeth reflect the geochemical signature of the region where the individual under study lived (Grupe et al., 1997; Bentley, 2006; Bentley and Knipper, 2005; Price et al., 2002).

Tooth enamel, used in this study, is not remodelled (in contrast to bone) and therefore preserves the isotopic signature of the environment in which the individual spent its childhood (or juvenile years, depending on the type of tooth analysed).

Kletthamer Feld is located in the geologically rather uniform pre-Alpine lowlands south of the Danube River (Fig. 3). Oligocene/Miocene marine sediments are covered by freshwater and loess deposits. The expected range for the carbonate area south of the Danube, which marks the border of the Roman
territory, lies at about 0.7080 to 0.7095 (Schweissing and Grupe, 2003a). This area south of the river has not been geologically mapped for Sr isotope ratios yet, but is characterized by values lower than 0.710 for granite (Grupe et al., 1997). Grupe et al. (1997) measured sediments from this area ranging between 0.70899 and 0.70992.

$^{87}\text{Sr}/^{86}\text{Sr}$ isotope analyses produced values between 0.70880 (EKF 1664) and 0.71007 (EKF 1665). All of the individuals show $^{87}\text{Sr}/^{86}\text{Sr}$ values lying within the local range except for one:

EKF 1665 (0.71007) (c. f. Table 3 and Fig. 4).

For comparison: Bentley and Knipper (2005) presented pig teeth from Altdorf-Aich, Inzigkofen, Mintraching, Wang and Wittislingen showing $^{87}\text{Sr}/^{86}\text{Sr}$ values (n = 8) with a rather narrow range of 0.7097 ± 0.0008. Specifically, the pig teeth from Wang (0.71046; 0.71033; 0.70964) and Altdorf (0.70982), 35 km and 43 km away from the Kletthamer Feld, respectively, present an average value of 0.71006. The authors admit the possibility that the pigs could have been traded. Assuming that the pigs were local, individual 1665 (0.71007) would be located at the upper limit of the possible local $^{87}\text{Sr}/^{86}\text{Sr}$ range.

The shortest distance to the granite area northeast of the Danube is 80 to 90 km, a distance that could be covered by walking or riding in 2 to 3 days. $^{87}\text{Sr}/^{86}\text{Sr}$ values slightly above the limit of 0.7100 might be the result of a mixture between granite-based and carbonate-based isotopic influence. This could be caused by moving from one geological region to another during childhood while tooth enamel formation is occurring. Another possibility is the consumption of food produced in both regions.

Thus the strontium data obtained from tooth material suggest one putatively mobile individual (EKF 1665) that might have lived in a different area during childhood years.
Assessment of family relationships by means of DNA analyses

It was possible to retrieve DNA from 11 out of 13 individuals and to reproduce unambiguous mtDNA profiles of the HVR I region for 9 individuals. Both samples for which no DNA could be obtained were of osseous material. Furthermore, sections of HVR I segment A could not be amplified or sequenced for samples 1700 and 1717, resulting in a limited degree of interpretability. Analyses of the second extract supported the results.

The purpose of the mtDNA analyses was to test whether some of the individuals were maternally related.

Table 5 shows the mtDNA profiles of the analysed individuals (eight different mtDNA-haplotypes could be observed) and the presumable assignments of haplogroups.

As the sequences were assembled out of four overlapping fragments, haplogroup allocations were checked by SNP-multiplex typing in order to exclude mosaic patterns, whereas the differentiation of H and non-H haplogroups was of special interest.

The SNP profiles for 6 of 9 individuals (2 individuals could not be typed) contained the C to T substitution at nucleotide position 7028, assigning these individuals as non-H (Macaulay et al., 1999). In all cases the sequencing results of the HVR I and the SNP-typing of the coding region were conform.

One female (EKF 1663) and two male individuals (EKF 1664 and 1704) display identical haplotypes. Given the small size of the burial site in combination with the low haplotype frequencies (although the latter can only be used to a restricted degree as they were calculated on the basis of recent data) the
Table 5 | Results of DNA analyses

<table>
<thead>
<tr>
<th>Sample</th>
<th>HVR I region</th>
<th>Frequency</th>
<th>Assumed haplogroup</th>
<th>Coding sequence</th>
<th>Macro-haplogroup</th>
<th>DYS3 91</th>
<th>DYS3 93</th>
<th>DYS19</th>
<th>Y-chromosomal STRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>EKF 1662</td>
<td>16126C, 16294T, 16296T, 16304C</td>
<td>1.6%</td>
<td>T2b</td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>13*</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>EKF 1663</td>
<td>16126C, 16163G, 16186T, 16189C, 16294T</td>
<td>1.3%</td>
<td>T1a</td>
<td>7028T</td>
<td>N</td>
<td>10</td>
<td>16</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>EKF 1664</td>
<td>16126C, 16163G, 16186T, 16189C, 16294T</td>
<td>1.3%</td>
<td>T1a</td>
<td>7028T</td>
<td>N</td>
<td>10</td>
<td>16</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>EKF 1665</td>
<td>No difference to rCRS</td>
<td>11%</td>
<td>H</td>
<td>No difference to rCRS</td>
<td>H</td>
<td>10</td>
<td>13*</td>
<td>23*</td>
<td>12</td>
</tr>
<tr>
<td>EKF 1699</td>
<td>16298C</td>
<td>1.6%</td>
<td>HV0</td>
<td>7028T</td>
<td>N</td>
<td>11</td>
<td>13*</td>
<td>14*</td>
<td>12</td>
</tr>
<tr>
<td>EKF 1700</td>
<td>16185C, 16189C, 16362C</td>
<td>1.6%</td>
<td>(K)</td>
<td>7028T</td>
<td>N</td>
<td>11</td>
<td>13</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>EKF 1703</td>
<td>16192T, 16256T, 16270T,</td>
<td>0.75%</td>
<td>U5a</td>
<td>7028T</td>
<td>N</td>
<td>10</td>
<td>16</td>
<td>15*</td>
<td>24</td>
</tr>
<tr>
<td>EKF 1704</td>
<td>16126C, 16163G, 16186T, 16189C, 16294T</td>
<td>1.3%</td>
<td>H1a</td>
<td>7028T</td>
<td>N</td>
<td>10</td>
<td>16</td>
<td>15*</td>
<td>24</td>
</tr>
<tr>
<td>EKF 1717</td>
<td>16124C, 16224C, 16311C</td>
<td>1.3%</td>
<td>(H)</td>
<td>–</td>
<td>–</td>
<td>10*</td>
<td>13*</td>
<td>15*</td>
<td>25*</td>
</tr>
<tr>
<td>EKF 1719</td>
<td>No difference to rCRS</td>
<td>11%</td>
<td>H</td>
<td>No difference to rCRS</td>
<td>H</td>
<td>10</td>
<td>13</td>
<td>14*</td>
<td>12</td>
</tr>
<tr>
<td>EKF 1720</td>
<td>16092C; 16140C; 16265G, 16265G, 16293G, 16311T</td>
<td>0.02%</td>
<td>H11</td>
<td>No difference to rCRS</td>
<td>H</td>
<td>10</td>
<td>13</td>
<td>14*</td>
<td>12</td>
</tr>
</tbody>
</table>

* = proven once only, () = parts of HVR I are missing, – not sure, blank field = no results
chances of a coincidental concordance seem to be small to none. Hence a maternal relationship can be assumed for those individuals.

The male individuals 1665 and 1719 also show equivalent DNA haplotypes. However, as they share today’s most common European haplotype, this provides only limited support for the assumption of a maternal relationship.

Maternal relationship can be excluded among the remaining individuals. Moreover, the mother of the only child in the group could not be detected. However, she might be one of the two females whose mtDNA sequences could not be successfully extracted.

Results of molecular sex typing via the amelogenin test could be obtained from 10 out of 13 individuals. Again the bone samples yielded no and sample 1720 inconclusive results. As can be seen in Table 1, the outcomes of morphological and molecular genetic sex typing match.

The analyses of Y chromosomal STRs revealed a pattern typical of ancient DNA, characterized by a decreasing rate of successful amplification inversely correlated with amplicon length of considered loci. While short-sized alleles like DYS391 and DYS393 (about 120bp) could mostly be amplified, the success rate grew weaker with increasing allele length with the amplification of locus DYS 389-II (about 380bp) producing only a few results.

Reproducible genotypes of four to eight loci could be retrieved from five individuals (EKF 1662, EKF 1664, EKF 1703, EKF 1704, EKF 1719). The rate of successful amplifications was lower for the remaining three individuals, mostly restricted to one PCR, which confirms the statements in respect of family relationships amongst them.

But the Y-STR pattern of male individuals 1664 and 1704 concurs and it could only be found in three out of 82251 analysed individuals from recent populations using the Y-STR haplotype reference database (www.yhrd.org). This consideration seems to make a paternal relationship quite likely. Because these individuals also had identical mt-haplotypes indicating maternal relations, they might be siblings.

The same situation is reflected in the findings of male individuals 1719 and 1665, where a maternal relation could be found and a paternal relationship cannot be excluded, due to potentially equivalent Y-STR profiles. Because of the incomplete patterns and the lack of reproducibility of some of the loci, this assumption cannot be completely verified though.

The remaining STR profiles differ in more than one locus so that affiliations to matching paternal lineages can be rejected. In total, six divergent Y-STR patterns could be observed.

In general, it is not possible to prove the authenticity of human aDNA (Pääbo et al., 2004). This is mainly due to the fact that human contamination can never be ruled out entirely. For this reason, the results of the present DNA analyses have to be considered and interpreted with reservation, despite all precautions taken to prevent contamination. There are certain points, however, that speak for the authenticity of the data:

- Systematic contamination of one or more skeletons can be ruled out, given the fact that none of the mtDNA haplotypes found are represented among the staff involved in morphological examination, sampling or laboratory work.
- All teeth selected for this work were still sitting firm in the alveoli. This guaranteed the best root protection possible during previous archaeological handling of the remains. Furthermore, the results of morphological sex typing concurred with the genetic sex typing, which also points to systemic contamination being highly unlikely.
- The primer-set for HVR I typing was used for the first time in our laboratory and no sample analyzed in any earlier project corresponded with the haplo- or genotypes of the Erding samples.
Extraction and PCR blanks were constantly negative. Therefore, intralaboratory contamination can be characterized as extremely unlikely.

- The observation of eight different and plausible haplotypes among eleven ancient individuals speaks for the authenticity of the results. If contaminant DNA was the source, it would seem highly unlikely that the remains of nearly all of the individuals were each contaminated with DNA of a different haplotype.
- The interpretation of the identical mitochondrial haplotype data and Y-STR profiles concerning samples EKF 1663, EKF 1664 and EKF 1704 as familial relationships and not as contamination is supported by the fact that one woman and two men shared this profile. Both the sex of the woman and the sex of the two men were correctly typed by molecular genetic means. For that reason it seems unlikely that the source of the presumed contaminating DNA is a male individual (which it would have to be because of the identical Y-STR patterns) who left only mitochondrial DNA and no chromosomal DNA, as would have had to be the case in view of the woman.
- The mtDNA HVR I sequences do not appear to be the mosaic results of combined, genetically unrelated fragments. The retrieved sequences can also be observed in recent populations and the HVR I profile-based assignments of haplogroups could be affirmed by SNP-typing of the coding region.
- The DNA analyses followed the expected patterns of appropriate molecular behaviour. This means no results could be obtained from bones, which are known to be more prone to degradation of DNA than teeth (Burger et al., 1997). In addition to that, the analyses of Y chromosomal STRs revealed a pattern typical of ancient DNA: decreasing rate of successful amplifications inversely correlated with single product lengths of considered loci. Furthermore the good preservation of another biomolecule under investigation, the collagen (see stable isotope quality criteria), generally also favours the preservation of DNA.

Conclusions

Archaeometric analyses were performed in order to validate or invalidate the previously postulated hypotheses. Considering the given results in conjunction with the morphological investigations, as well as the historical context, the following statements can be made:

The hypothesis of the founder population was the only one that could be clearly rejected. On the basis of the isotopic data, we can rule out the possibility that the buried individuals were pioneers. None of the eleven individuals spent his/her childhood in an area with a differing Sr signature. Only one individual showed slight evidence for mobility during early childhood.

It must be pointed out that strontium analysis does not allow the detection of immigrants from regions that display a signature that is geochemically identical to the local signature. But if the skeletal population of the Kletthamer Feld was a Germanic founder population, their provenance would have to be from a region beyond the Danube, which separated the Roman Empire from the barbarians in the north. The nearer northern regions are characterized by distinctly higher Sr signatures, though. For this reason, Germanic immigration from northern border regions can very probably be ruled out.

Furthermore, stable isotopes of light elements, which provide an indication of diet, climatic and environmental conditions of the final years of an individual’s life, were analysed. Here, too, the majority of the samples produced values that concur with those one would expect to obtain for the area of the burial
site. Interestingly, three out of the four females (EKF 1702, EKF 1663; EKF 1721), but none of the males, display distinctive features.

Given the relatively slow remodelling rates of bone (mineral 3%/year; organics 24%/year; Dempster, 1999; Price et al., 2000) and considering the relatively early ages of death (adult) of these three women, these findings could suggest that the individuals in question spent parts of their adolescence in territories with different climates and settled down in Erding only in the final years of their lives. As the individuals in question were classified exclusively as females, this might be an indication of an exogamous marriage system, a phenomenon which has also been described in other studies analysing patterns of exogamous female mobility during the Late Roman and Early Medieval (Hakenbeck et al., 2010).

Due to the bone remodelling rates, differing signatures can be detected only for those women who died within their first years of marriage and hence shortly after their change of location.

This would explain why EKF 1700 is the only female who showed no irregularities regarding the isotopic ratios of the light elements pointing to immigration, as she lived to a mature or even to senile age. There would, then, have been enough time for her bones to remodel after a potential marriage and adjust the isotopic composition of a foreign territory.

Thus, it is unlikely that the skeletal remains belong to a newly established society in Erding; rather, they appear to represent a male population invariable in territory with potential female immigration.

This interpretation makes evident the complexity of migration: it cannot always be recognized by strontium analyses alone but rather requires a combination of multiple isotopic systems, as well as morphological traits such as age at death and sex.

Unlike the females, the majority of the men died at a mature age. It must be pointed out that differing climatic conditions experienced during adolescent years – hence before a potential relocation to this area – would remain unrecognised as the bones of these individuals would have had sufficient time to remodel and adjust their isotopic composition, as may have been the case with the female individual 1700.

The second theory, which described the buried population as a single small-sized family who administered an estate situated near the burial place, can only be partially rejected.

Relational ties are more than likely between at least three individuals. There are two men (EKF 1664 and 1704) whose common maternal and paternal lineage point to their relationship as siblings. These men can be associated with Individual 1663, probably a female.

Furthermore, there are indications suggesting maternal and paternal relationships between the males 1665 and 1719. However, the DNA data have to be considered with caution.

The great variability, in particular that detected with respect to paternal lineages (8 individuals, 6–7 different haplotypes), speaks against the identity of the buried people as members of an extended family. An exogamous marriage system would explain the also large variability of the maternal lineages (11 individuals, 8 haplotypes) but not that of the paternal lineages. So the interred individuals most probably do not belong to a single family clan. However, one cannot rule out the possibility that one or multiple nuclear families who managed one or various farms nearby were buried here with their servants.

However, the pattern of family relationships revealed in the analyses also fits in with the third hypothesis – that the buried men were members of a Roman military unit who lived together with their families near the burial place. In addition to the finding of the Roman fibula, the observed preponderance of men and the diversity of the Y-haplotypes support this supposition. EKF 1665 is an exception though, displaying Sr values that differ slightly from the local signature.
Overall, however, the results obtained best support the hypothesis that the buried individuals were members of a military unit and their families. Direct affirmation by archaeometric analyses is not possible in this case though.

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References


