Tracing mother-child relations in Austrian Early Bronze Age communities through mitochondrial DNA

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Zusammenfassung

Mutter-Kind-Beziehungen in österreichischen Gemeinschaften der frühen Bronzezeit im Spiegel mitochondrialer DNA

Die Kombination aus archäologischer Kontextanalyse und antiken genetischen Daten liefert wichtige Einblicke in das soziale Gefüge der Bronzezeit, einschließlich Verwandtschaft, persönliche und emotionale Verbindungen sowie den sozialen Status von Einzelpersonen innerhalb von Gemeinschaften. In diesem Beitrag untersuchen wir Mutter-Kind- und andere Familienbeziehungen in frühbronzezeitlichen Doppel- und Mehrfachbestattungen aus Niederösterreich (ca. 2200–1600 v.Chr.). Soziale und emotionale Verbindungen können aus der Art und Weise abgeleitet werden, wie Körper zueinander in Beziehung gesetzt, nach dem Tod gehandhabt und behandelt wurden sowie aus den in den Gräbern platzierten Gegenständen, die der Nahrungsaufnahme und Pflege dienen sollten. In sechs von elf untersuchten archäologischen Kontexten wurde eine enge genetische Verwandtschaft durch mitochondriale DNA festgestellt. Frauen und Kinder, die gemeinsam begraben wurden, waren jedoch nicht unbedingt verwandt. Dies könnte darauf hindeuten, dass die Ideologie von Mutterschaft nicht ausschließlich mit biologischen Müttern in Verbindung gebracht wurde und Kinderbetreuung gemeinschaftlich organisiert war.

Summary

The combination of archaeological context analysis and ancient genetic data provides important insights into the social fabric of the Bronze Age, including kinship, personal and emotive connections, and the social status of individuals within communities. In this contribution, we investigate mother-child and other family relationships in Early Bronze Age double and multiple burials from Lower Austria (c. 2200–1600 BC). Social and emotional links may be inferred from the ways bodies were placed in relation to each other, handled and treated after death, and through objects placed in the graves intended for feeding and care. A close genetic relationship through mitochondrial DNA was found in six out of 11 investigated archaeological contexts. Women and children buried together, however, were not necessarily related. This might suggest that the ideology of mothering was not exclusively associated with biological mothers and caregiving was organised communally.

Introduction

In recent years, the interest in exploring motherhood in history and prehistory has increased, with cultural-historical or social archaeological approaches[1], as well as bio-archaeological, evolutionary, isotopic and genetic approaches to motherhood on the rise (Gowland/Halcrow 2020; Hassett 2022; Rebay-Salisbury et al. 2018). In addition to the biological framework of sexual reproduction, motherhood includes a range of cultural choices and practices that are fruitful to explore for a better understanding of motherhood in the past.

Whereas researching motherhood tackles both the biological aspect of pregnancy, parturition and breastfeeding and the ideological and social aspects of childcare embedded in the social framework (Rich 1995), mothering is a term used for the childcare practices associated with mothers. Mothers traditionally, historically, and still today predominantly perform childcare, whereas fathers, older siblings and grandparents may help or substitute at times; childcare is thus strongly associated with women's social roles (Spencer-Wood/Seifert in press).

To what extent biological relatedness underpins mother-child relations varies between cultures and has yet to be explored for Central European Bronze Age communities. Kinship in general is comprised of a web of relations through a range of social mechanisms, including descent and marriage, as well as adoption and friendship (Chapais 2008; Stone 2009). The importance of genetic relatedness to the construction of kinship is hotly debated amongst anthropologists[2], and equally important to explore archaeologically (Johnson/Paul 2016).

Cemeteries, or other archaeological contexts in which individuals are buried together in close bodily contact, are particularly well suited for an analysis of kinship. Regardless of

1 Cooper/Phelan 2017; Hackworth Petersen/Salzman-Mitchell 2012; Myers 2017; O'Reilly 2014; Romero/López 2018.

2 E.g. Carsten 2003; Sahlins 2011; 2011a; Schneider 1984; Shapiro 2018.

  (HTTPS://DOI.ORG/10.11588/PROPYLAEUM.1280.C18009)

the cause of death, they imply relationships of kinship, familiarity or affection between the individuals. A woman buried with a child is often assumed to be the mother – be it biological or social – but infants may also be included in graves for reasons other than relatedness, e.g., similar time of death, convenience, or protection. Testing the relationship between women and children buried together may reveal if, and to what extent, social concepts of motherhood implied a genetic link and whether childcare was organised individually or communally.

Mitochondrial DNA analysis

Within the framework of the project ›The value of mothers to society: responses to motherhood and child-rearing practices in prehistoric Europe‹, we applied mtDNA analysis to 28 individuals from 11 selected Early Bronze Age (c. 2200–1600 BC) burial contexts in Lower Austria to clarify their genetic relationship. Since mtDNA is normally inherited from the mother only, it is particularly suited to investigate mother-child relationships – a mismatch rules out a maternal relationship. In addition, mtDNA is present in multiple copies per cell, which makes it more likely to produce results in small and degraded samples (Parson et al. 2018; Rott et al. 2018).

Minimising damage to the collections curated by the Anthropological Department of the Natural History Museum in Vienna was the guiding principle in our sampling strategy. Originally, we aimed at obtaining two 100–150 mg samples per individual to optimise the chances of yielding DNA suitable for analysis. From 2015–2017 we primarily targeted petrous bone, femur and teeth in individuals from Franzhausen, Unterhautzenthal and Zwingendorf. Targeting the osseous inner ear inside the petrous pyramid appears to return the highest endogenous aDNA yields (Pinhasi et al. 2015), but it is present only twice in the body, and then only when recovery of the whole body was possible.

After running the first batch of analysis, it became clear that samples from the pulp area of deciduous and permanent teeth are best suited. For the second batch of samples from Schleinbach, therefore, we selected only one tooth per individual. For those solid tissue samples (teeth and bones) that contained enough starting material, two series of analyses based on milled and drilled powder were performed. Otherwise, only one of the methods was applied. The analysis followed the protocol described in Parson et al. (2018).

In brief, the surfaces of the remains were initially mechanically treated using a Dremel tool and subsequently chemically cleaned using a 5 % bleach solution. The cleaned surfaces were then drilled with a dental drill at low speed to prevent overheating and/or milling was performed in a Retsch grinding mill MM400 (Retsch GmbH, Haan, North-Rhine Westphalia, Germany). A total of 50 mg of the resulting powder was lysed and DNA was extracted following the modified Dabney protocol described in Xavier et al. (2021). MtDNA-specific quantitation was performed using *SD quants* (Xavier et al. 2019) that co-amplify a nuclear target (70 bp), two differently sized mtDNA targets (69 and 143 bp) and an internal PCR control to test for inhibition in a tetraplex real-time PCR quantitation system. Those samples that provided the highest mtDNA quantities were further subjected to mtDNA sequence library preparation using the IonXpress Fragment Library Kit (ThermoFisher Scientific, Waltham, Massachusetts, USA; *TFS*) according to the protocol described in Eduardoff et al. (2017). Massively Parallel Sequencing was performed on an Ion S5 (*TFS*) with automated template preparation using the IonChef pipeline (*TFS*) according to the manufacturer's protocol. Raw data were analysed as described in Strobl et al. (2019). MtDNA consensus haplotypes were aligned relative to their known phylogenetic neighbours following the concept outlined in Bandelt/Parson (2008) and reported relative to the revised Cambridge Reference Sequence (Andrews et al. 1999), as outlined in Parson et al. (2014). Polynucleotide stretches were ignored for interpreting the haplotypes. Haplogroups were estimated using SAM2 (Huber et al. 2018) based on Phylotree (mt) haplogroup nomenclature (van Oven/Kayser 2009), using the haplogrouping function in EMPOP (Parson/Dur 2007).

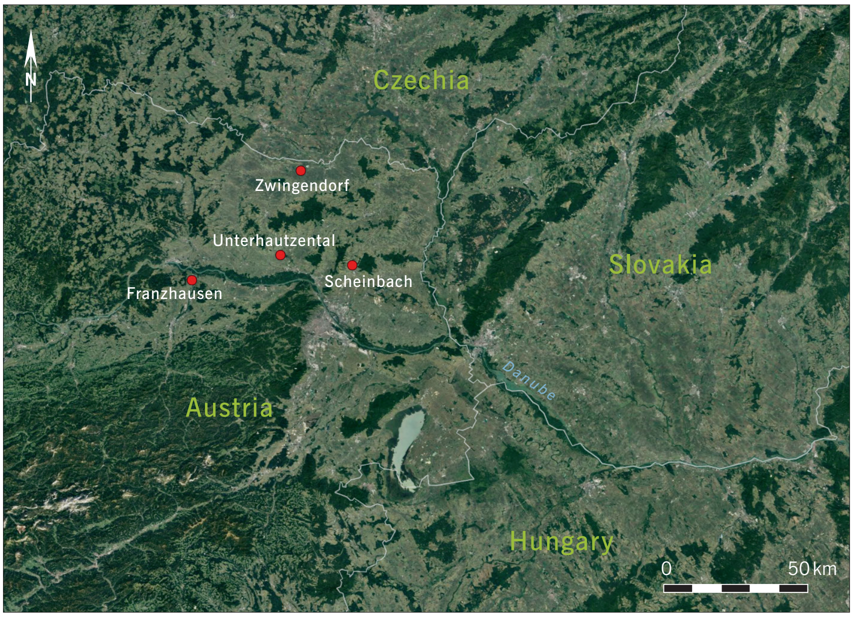

Fig. 1 Map of the study region with the Early Bronze Age sites of Franzhausen, Schleinbach, Unterhautzenthal and Zwingendorf in today's Austria.

Abb. 1 *Karte des Untersuchungsgebiets mit den frühbronzezeitlichen Fundstellen Franzhausen, Schleinbach, Unterhautzenthal und Zwingendorf im heutigen Österreich.*

Site	Context	Individual	NHM AA Inv. Nr.	Grinding Method	Quant.		
					nDNA (70 bp) pg/µl	143 bp mtGE/µl	69 bp mtGE/µl
Zwingendorf							
1	Grave 8	8A	–	drill	423.0	214.6	1556.3
2		8B	–	mill	0.9	216.4	2101.2
Schleinbach							
3	Grave 30/31	30	27635	mill	0.3	2.9	147.7
4		31	27636	mill	0.8	21.6	305.1
5	Pit 60	60	27642	mill	1.0	14.0	98.4
6		60A	27643	drill	0.2	5.4	188.8
7		60B	27644	drill	0.2	16.8	197.0
8		60C	27645	drill	0.1	4.6	102.9
Franzhausen							
9	Grave 599	599A	24076	drill	1.3	18.2	316.8
10		599B	24077	drill	10.7	4.6	166.0
11		599C	24078	drill	46.8	31.2	651.9
12	Grave 860	860/4	24183	drill	0.2	358.0	6346.9
13		860/1+2	24182	drill	0.4	0.7	20.5
14		860/3	24182	drill	0.2	69.9	1352.7
Unterhautzenthal							
15	Pit 27	27A	24904	drill	0.4	473.6	2634.0
16		27B	24905	mill	1.3	113.9	1101.7
17	Grave 38	38A	24908	mill	0.2	NA	4.3
18		38B	24908	drill	0.3	0.5	1.4
19	Grave 95	95	24921	mill	1.5	7.3	55.5
20		95A	24922	mill	0.3	2.8	46.3
21		95B	24923	mill	0.8	1.9	9.7
22	Grave 103	103A	24929	mill	8.3	9.5	171.8
23		103B	24929	mill	0.4	0.3	0.9
24	Grave 109	109A	24933	mill	0.6	0.5	8.2
25		109B	24933	mill	11.5	178.0	1124.3
26	Grave 122	122A	24937	drill	0.9	1.1	6.7
27		122B	24938	mill	2.2	2.1	41.3
28		122C	24938	mill	0.4	0.2	0.5

Tab. 1 Summary of nuclear DNA (70 bp target) and mitochondrial DNA (143 and 69 bp targets) quantitation results.

Tab. 1 *Zusammenfassung der Quantifizierungsergebnisse für Kern-DNA (70-bp-Fragment) und mitochondriale DNA (143- und 69-bp-Fragmente).*

DNA preservation

The solid tissue samples were subjected to both milling and drilling methods, depending on the amount and nature of the available tissue (Tab. 1). Nuclear DNA quantities (70 bp target) were found between 0.2 and 423 pg/µl; mtDNA quantities ranged between 0.4 and 473 copies/µl and 0.5 and 6346 copies/µl for the 143 bp and the 69 bp target, respectively. The extracts contained roughly 10–20 times more of the 69 bp mtDNA template compared to the 143 bp mtDNA target, which reveals and confirms the expected degraded state of the mtDNA extracted from the ancient tissues. This result suggested the application of capture-based mtDNA typing (Primer Extension Capture, *PEC*; see Eduardoff et al. 2017) instead of PCR-based schemes that require larger DNA templates. Samples 18, 23 and 28 were not further sequenced due to the low mtDNA quantities.

We were able to obtain interpretable mtDNA results from 34 of 46 samples (74 %) and 25 of 28 (89 %) tested individuals (Tab. 2). DNA preservation was found to be highest in the pulp cavity of the teeth (all successful) and lower for petrous bone (62 %) and femur (60 %). We only took two samples from ribs and one each from a humerus and tibia. The humerus appeared green, apparently due to copper salts from bronze

Site	Context	Individual	Age	Sex	Haplogroup	Haplotype	EMPOP Query (reduced to CR)*
Zwingendorf							
1	Grave 8	8A	14–16	—	K1a4b[1]	73G 263G 280G 315.1C 497T 524.1A 524.2C 750G 3480G 8860G 11299C 11719A 12308G 12372A 13590A 14167T 14766T 14798C 15326G 16093C 16224C 16311C 16519C	none in 38361/15782
2		8B	4	—	K1a4b[2]	73G 263G 280G 315.1C 497T 524.1A 524.2C 750G 1189C 1438G 1811G 2706G 3480G 4769G 6026A 6899A 7028T 8029T 8860G 9055A 9448G 9698C 10398G 10550G 11299C 11467G 11485C 11719A 12308G 12372A 13590A 14167T 14766T 14798C 15326G 16093C 16224C 16311C 16519C	none in 38361/15782
Schleinbach							
3	Grave 30/31	30	30–35	♂	H+152[3]	152C 263G 315.1C 750G (4769G) 8860G 15326G 16519C	162 in 38361/ 129 in 15782
4		31	27–30	♂	H+152[4]	152C 263G 315.1C 750G 4769G 8860G 15326G 16519C	162 in 38361/ 129 in 15782
5	Pit 60	60	30–35	♂	H2a1[5]	263G 315.1C 750G 8860G 16354T	30 in 38361/ 23 in 15782
6		60A	8–9	♂**	U2e3 (U2e3a)[6]	73G 152C 217C 263G 315.1C 394T 508G 524.1A 524.2C 750G 1811G 3170A 4769G 5390G 5426C 8860G 10876G 11719A 12308G 12372A 13020C 13734C 14180C 14766T 15326G 15721C 15907G 16051G 16129C (16182M) 16183C 16189C 16260T 16356C 16362C 16519C	none in 38361/15782
7		60B	12	♀**	J1c2[7]	73G 185A 188G 228A 263G 295T 315.1C 462T 489C 750G 14766T 14798C 16069T 16126C 16519C	52 in 38361/ 40 in 15782
8		60C	3–4	♀**	U2e3 (U2e3a)[8]	73G 152C 217C 263G 315.1C 394T 508G 524.1A 524.2C 750G 5390G 5426C (10876G) (11467G) (12372A) 13734C 15907G 16051G 16129C (16182M) 16183C 16189C 16260T 16356C 16362C 16519C	none in 38361/15782
Franzhausen							
9	Grave 599	599A	20–25	♂	T2e[9]	73G 150T 195C 263G 315.1C 709A 750G 1888A 2706G 4216C 4769G 4917G 8697A 8860G 10463C 11251G 11719A 11812G 13368A 14233G 14766T 14905A 15326G 15452A 15607G 15928A 16126C 16153A 16294T 16296T 16519C	1 in 38361/ 1 in 15782
10		599B	14–16	—	R1[10]	54C 73G 146C 150T 152C 189G 195C 241G 263G 315.1C 750G 15940C 16235G 16311C 16519C	none in 38361/15782
11		599C	12–14	—	H[11]	263G 315.1C 750G 16519C	791 in 38361/ 630 in 15782
12	Grave 860	860/4	3–5	♀**	K1a4a1i[12]	73G 263G 309.1C 315.1C 497T 524.1A 524.2C 573.1C 750G 9698C 11299C 12308G 12372A 13740C 14167T 14766T 14798C 15880G 16224C 16311C 16519C	15 in 38361/ 12 in 15782
13		860/1+2	35–50	♀	U5a1d2a[13]	73G 195C 263G 315.1C 573.1C 750G 16145A 16189C 16192T 16256T 16270T 16399G	5 in 38361/ 5 in 15782
14		860/3	7–8	—	U5a1d2a[14]	73G 195C 263G 309.1C 315.1C 573.1C 750G 8860G 12372A 13617C 14766T 14793G 16145A 16189C 16192T 16256T 16270T 16399G	5 in 38361/ 5 in 15782
Unterhautzenthal							
15	Pit 27	27A	2	—	H5a1[15]	263G 315.1C 456T 523del 524del 750G 4769G 4336C 8860G 15326G 15833T 16129A 16183C 16189C 16304C	none in 38361/15782

Site	Context	Individual	Age	Sex	Haplogroup	Haplotype	EMPOP Query (reduced to CR)*
16		27B	6	—	H5a1[16]	263G 315.1C 456T 523del 524del 750G 4336C 4769G 8860G 15326G 15833T 16129A 16183C 16189C 16304C	none in 38361/15782
17	Grave 38	38A	14–15	—	T2e[17]	263G 315.1C 709A 750G 15928A 16126C 16153A 16519C	1 in 38361/ 0 in 15782
18		38B	0–0.3	—		NA	
19	Grave 95	95	35–45	♀	HV[18]	150T 152C 263G 315.1C 750G 8860G 16222T 16519C	none in 38361/15782
20		95A	3–4	♀**	T2[19]	73G 207A 263G 315.1C 709A 750G 8697A 15928A 16126C 16294T 16296T 16519C	none in 38361/15782
21		95B	4–5	♂**	T2[20]	73G 207A 263G 315.1C 709A 750G 794C 15928A 16126C 16294T 16296T 16519C	none in 38361/15782
22	Grave 103	103A	16–20	♀	U5b[21]	73G 150T 263G 315.1C 750G 12308G 12372A 14182C 15326G 16147T 16189C 16270T	1 in 38361/ 1 in 15782
23		103B	2.5–3.5	—		NA	
24	Grave 109	109A	40–60	♀	U2e1[22]	73G 152C 217C 263G 315.1C 340T 508G 750G 15907G 16051G 16129C 16189C 16362C 16519C	none in 38361/15782
25		109B	0–0.2	—	J1c1[23]	73G 185A 228A 263G 295T 315.1C 462T 482C 489C 750G 3394C 4216C 4769G 8860G 11251G 11719A 13708A 14766T 14798C 15326G 15452A 16069T 16126C 16352C	none in 38361/15782
26	Grave 122	122A	35–40	♂	I2'3[24]	73G 152C 199C 204C 207A 250C 263G 315.1C 750G 15924G 16129A 16223T 16391A 16519C	22 in 38361/ 18 in 15782
27		122B	17–20	♀	T1a1'3[25]	73G 152C 195C 263G 315.1C 709A 750G 15928A 16126C 16163G 16186T 16189C 16294T 16519C	136 in 38361/ 113 in 15782
28		122C	0.25–0.5	—		NA	

1 1–982 3434–3575 5059–5541 8126–9007 10731–11402 11712–12477 13499–14873 15285–16569
2 1–16569
3 1–907 1652–2100 3392–3924 3967–4524 4592–4795 4848–5670 5854–6005 6351–6624 7268–7982 8157–9263 9364–9696 9777–9916 10734–11367 11485–12676 12787–12986 13027–14946 15262–15732 15764–16569
4 1–930 1694–1757 1816–1950 2051–2374 3419–3604 3765–3912 4123–4246 4639–4790 5019–5715 6131–6260 6405–6630 7343–7499 8031–8989 9207–9321 9505–9715 10173–10332 10681–11368 11922–12186 12255–12620 13059–14944 15315–16569
5 1–950 8220–8581 8775–8953 10833–10962 15764–16569
6 1–882 1618–1757 1793–1974 2149–2463 2750–2890 3150–3622 3765–3897 3983–4307 4362–4531 4599–4782 4894–5665 6392–6660 7319–7510 7645–7911 8167–9017 9090–9692 10083–10319 10766–11397 11514–11761 11902–12575 12835–15080 15273–16569
7 1–897 8195–8709 14727–14854 15744–16569
8 1–900 5109–5461 8217–8749 10853–10973 11220–11355 11425–11555 12036–12235 12351–12424 13724–13888 15787–16569
9 1–978 1616–2490 2670–2758 3173–3621 3725–4287 4369–5728 6363–6702 8130–9693 10204–12594 12792–14989 15250–16569
10 9–897 8187–8701 15704–16569
11 10–978 8098–8773 15663–16569
12 1–948 8153–8817 9490–9731 10701–11342 12285–12504 13610–14878 15715–16569
13 1–862 2181–2390 8156–8466 8521–8679 10782–11041 13760–14615 15699–16569
14 1–924 8101–8986 12310–12567 13277–14830 15605–16569
15 1–1017 1570–2619 3071–6701 7307–10397 10479–15081 15187–16569
16 1–1283 1601–2522 3095–5771 5860–6707 7288–15040 15207–16569
17 216–519 602–802 15821–16270 16406–16567
18 1–901 8207–9073 15811–16569
19 1–841 8273–8440 8582–8711 15816–16569
20 20–460 557–865 15799–16569
21 1–954 8097–8785 10609–11389 11904–12537 14060–14697 15300–16569
22 1–950 8192–8465 15718–16569
23 1–1075 1589–1980 2100–2415 3271–5521 6273–6660 7397–9730 10581–11758 12824–14985 15259–16569
24 1–862 8276–8698 15809–16569
25 1–935 8194–8537 10813–11036 15700–16569

* Haplotypes were restricted to the control region (16024-576) for EMPOP frequency query purposes; Total numer of samples in EMPOP: 38361; number of samples belonging to Westeurasian Metapopulation: 15782.
** Sex identified by nanoflow liquid chromatography-tandem mass spectrometry of sexually dimorphic amelogenin protein fragments in dental enamel.

Tab. 2 Summary of mtDNA haplotype results and estimated haplogroups based on the successful sequencing ranges.

Tab. 2 *Zusammenfassung der mtDNA-Haplotyp-Ergebnisse und geschätzten Haplogruppen basierend auf den erfolgreichen Sequenzierungsbereichen.*

grave goods that had stained the bone; this sample did not return results. In addition to the tissue choice, the age of the individuals seemed to affect DNA preservation. The only individuals that did not return interpretable results included the foetus from Unterhautzenthal 38, the neonate from Unterhautzenthal 122 and the 2.5–3.5-year-old from Unterhautzenthal 103 (Tab. 2). The limited sample size for sub-adults may be an additional reason why these samples did not return results.

Early Bronze Age burial practices in the Lower Austrian study region (c. 2200–1600 BC)

The Danube separates two distinct early Bronze Age cultural groups in Lower Austria that differ by the way they buried their dead (cf. Fig. 1). The area north of the Danube is part of the Únětice complex, where male and female bodies were most often placed in flexed positions on their right side, with the head to the south and looking towards the

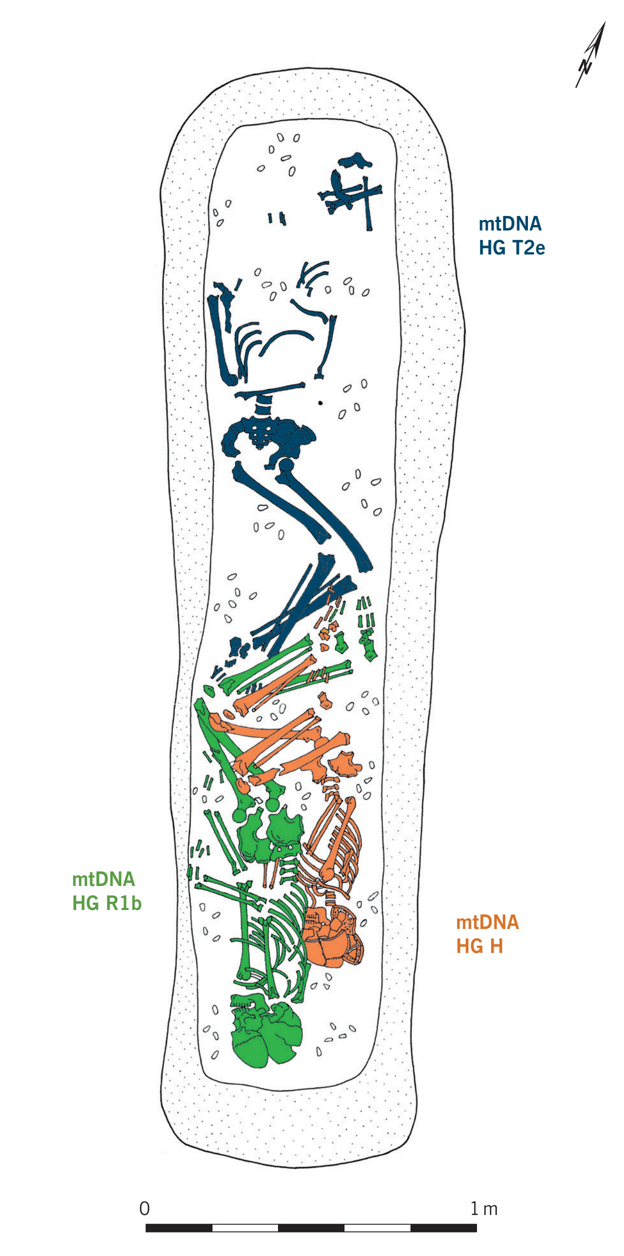

Fig. 2 Triple grave 599 at Franzhausen I, Austria, with the bodies of a 20–25-year-old male individual and two adolescents, aged 14–16 and 12–14.

Abb. 2 *Dreifachgrab 599 aus Franzhausen I, Österreich, mit den Skelettresten eines 20–25-jährigen Mannes und zweier Jugendlicher im Alter von 14–16 und 12–14 Jahren.*

east. Graves with single burials were the norm, but double and multiple burials were frequent; our study includes burials from Schleinbach (Pany-Kucera et al. 2020), Unterhautzenthal (Lauermann 1991; Lauermann 1995; Rebay-Salisbury et al. 2018b) and Zwingendorf (Wewerka 1982). Cemeteries of the Únětice complex are usually located near settlements in Lower Austria and comprise a dozen to several dozen graves (Lauermann 2003).

South of the Danube and west of the Viennese woods, the Unterwölbing Group connects to the Straubing Group farther west. In this region, burials were strongly gendered, even for children (Rebay-Salisbury et al. 2022a). Men were normally placed in a flexed position on their left side with the head to the north, women were usually positioned on their right side with the head to the south – both looked towards the east. Cemeteries may include hundreds of individuals, such as at Franzhausen I and II, but double and multiple burials are rare (Neugebauer/Neugebauer 1997; Neugebauer 1991). Social differences were marked by the depth of graves and the inclusion of bronze and other grave goods; the re-opening of graves and removal of objects was very common (Sprenger 1999).

Franzhausen

The cemeteries of Franzhausen I (Neugebauer/Neugebauer 1997) and II (unpublished) in the Traisen valley, one of the most important archaeological landscapes of Lower Austria, encompass over 2200 burials. Only four of the 716 excavated graves at Franzhausen I contained double or multiple burials. The triple burial 599 in a narrow wooden coffin held the remains of a 20–25-year-old male individual (599A) placed on the left side, head north-west; the upper part of the body was found severely disturbed and the cranium of the man was missing. Two young individuals, 14–16 (599B) and 12–14 (599C) years at death, were also placed on the left side of their bodies, but head south-east, at the feet of the adult, so that the lower legs came to rest in close contact. The man had been placed in the coffin first, followed by the older adolescent; the youngest was placed behind the older one's back in a single act of deposition (Rebay-Salisbury 2018). We found no indication of maternal relatedness through mtDNA in Grave 599 and can exclude siblingship via the mother; the individuals may, of course, have had the same father (Fig. 2).

In the ten documented consecutive graves of Franzhausen, burials were found on top of each other within the same grave pit and frequently with a substantial build-up of soil between the bodies. Grave 860 contained the body of a 3–5-year old girl (860/4) who was buried first; the burial was found severely disturbed, but the location of the bowl and skull indicated a female burial (confirmed by peptide analysis of dental enamel, see Rebay-Salisbury et al. 2022a). The remains of a 35–50-year-old woman (860/1+2) and a 7–8-year-old child (860/3) were found 117 cm higher up in the grave. The excavators suggested that these were the bodies of two individuals that had been removed from other graves in the course of grave robbing whilst the bones were still partly articulated (Neugebauer/Neugebauer 1997). Testing if and what kinds of kinship relations existed between these individuals also contributes to the debate of whether grave re-opening in the Early Bronze Age served primarily material gain, perhaps even from groups unrelated to the ones burying the dead (Jankuhn 1978; Neugebauer 1988), or whether it was a socially accepted ritual practice within the community (Aspöck et al. 2020; Rittershofer 1987).

Through mtDNA analysis, we found no maternal relatedness between the 3–4-year-old girl buried first and the other two individuals buried over a meter higher up in the same grave shaft in Grave 860. The other two individuals, a 35–50-year-old female and a 7–8-year-old child, however, share the same haplotype assigned to haplogroup U5a1d2a. This is remarkable, as the upper two individuals are thought to be secondary deposits within this grave (Neugebauer/Neugebauer 1997). The results of the mtDNA analysis indicate that relatedness was a factor in the final deposition even for multi-staged burials.

Unterhautzenthal

Unterhautzenthal is an Únětice Culture site with settlement features and graves of 57 individuals (Lauermann 1991; Lauermann 1995; Rettenbacher 2004). Five burials of women with children had been discovered at the site and the skeletal preservation was excellent, which is why it served as a pilot study for our work on pelvic modifications through pregnancy and childbirth (Pany-Kucera et al. 2019; Rebay-Salisbury et al. 2018).

Grave 95 included the burial of a 35–45-year-old woman adorned with some twisted bronze wire hair rings and buried in a flexed position on the right side of their body; directly in front of her face, a 3–4-year-old girl[3] wearing a bronze neck ring and a dress pin was placed in a similar position. The heads of both individuals were covered by a large ceramic bowl. Behind the legs of the adult woman, a 4–5-year-old boy was deposited wearing a bronze bracelet. Seven complete ceramic vessels and fragments of other vessels had been placed around the individuals in the grave. Despite being buried in close physical contact, mtDNA analysis was able to rule out a biological mother-child relationship: whilst the children share a T2 mitotype, the female's mtDNA belongs to HV. The burial context and the placing of the individuals nevertheless suggest social kinship between the adult and the children, who could have been siblings. The closest possible relation based on the age difference between the persons buried together is that the woman was their paternal grandmother; she might have been a member of the father's kin or entirely unrelated (Fig. 3).

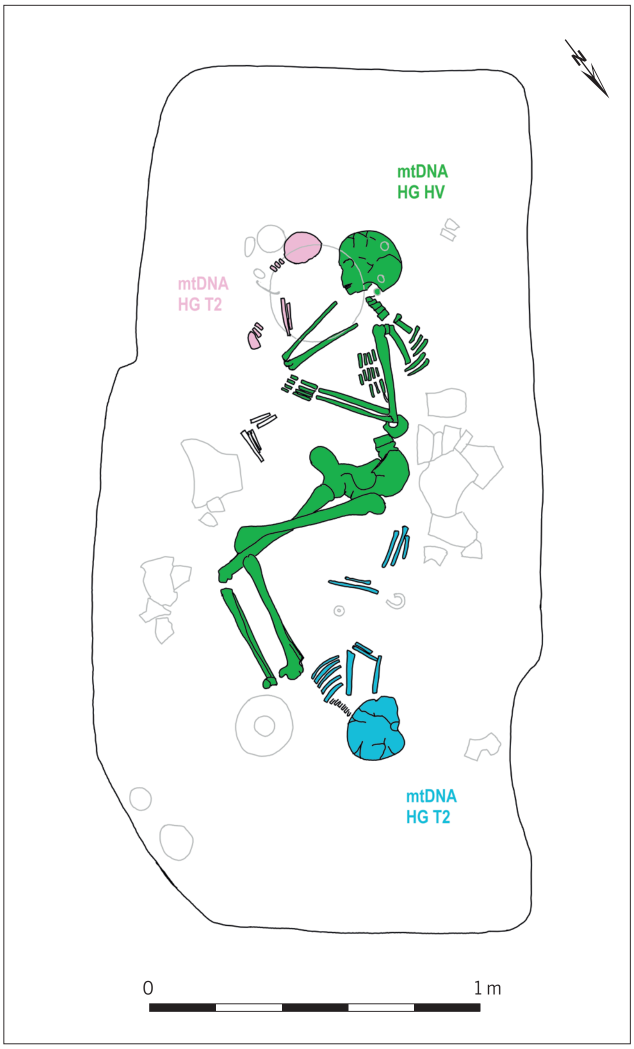

Fig. 3 Grave 95 from Unterhautzenthal, Austria, with the bodies of a 35–45-year-old woman buried with a 3–4-year-old girl and a 4–5-year-old boy.

Abb. 3 *Grab 95 aus Unterhautzenthal, Österreich, mit den Skelettresten einer 35–45-jährigen Frau, bestattet mit einem 3–4-jährigen Mädchen und einem 4–5-jährigen Jungen.*

The body of a 50–60-year-old female buried in a flexed position on the right side of their body and adorned with jewellery, a bronze dress pin and ten twisted bronze wire hair rings, was deposited in Grave 109. The pottery set included a decorated cup, a jug, and a bowl. This grave also contained the left femur of a neonate. The mtDNA lines of the two individuals are entirely unrelated (U2e1 and J1c1), which clarified that the woman was not the newborn's mother. Single human body parts, particularly of foetuses and small children, are not uncommon in Early Bronze Age grave fill; it remains unclear if they were deliberate additions to the grave assemblages.

A foetus/neonate of 34–36 weeks gestational age was found with a 14–15-year-old adolescent in Grave 38, buried with the legs tightly flexed towards the right. The grave contained three bowls, a handled cup and a jug, and was spacious in relation to the size of the body.

Remains of a slightly older child, estimated at between 2.5 and 5.5 years at death, comprised fragments of a *scapula*, *humerus* and *ulna*. They accompanied the 16–20-year old in Grave 103, whose body was found in a loosely flexed position with legs tilted to the right. A bronze pin and two small bronze rings were found in the chest area; a pottery set with a large bowl and three small cups as well as a bone awl and a chipped stone tool were placed at the feet.

Grave 122, the double burial of a 35–45-year-old male and a 17–20-year-old female, included a 3–6-month-old infant. With both adults' bodies placed in flexed positions on the right side of their body, the woman lay in front of the man; it is unclear where the remains (cranial fragments and *tibia*) of the infant were found. The burials were heavily disturbed, but the remnants of bronze jewellery and pottery suggest a well-furnished grave.

We were not able to confirm or reject the hypothesis that the female individuals in graves 38, 103 and 122 were the mothers of the foetuses or newborns whose remains they

3 The sex of the children was determined by sexually dimorphic amelogenin protein fragments in dental tooth enamel by nanoflow liquid chromatography-tandem mass spectrometry (Rebay-Salisbury et al. 2020; Rebay-Salisbury et al. 2022a).

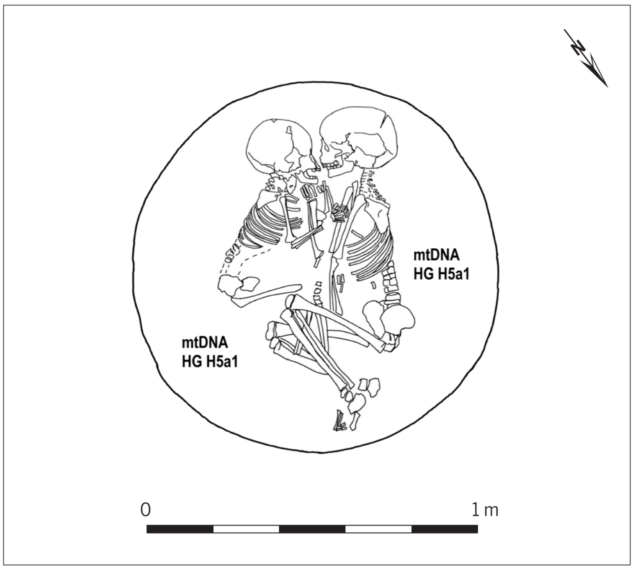

Fig. 4 Burial of a 2- and a 6-year-old child in settlement pit 27 from Unterhautzenthal, Austria.

Abb. 4 *Bestattung eines 2- und eines 6-jährigen Kindes in der Siedlungsgrube 27 aus Unterhautzenthal, Österreich.*

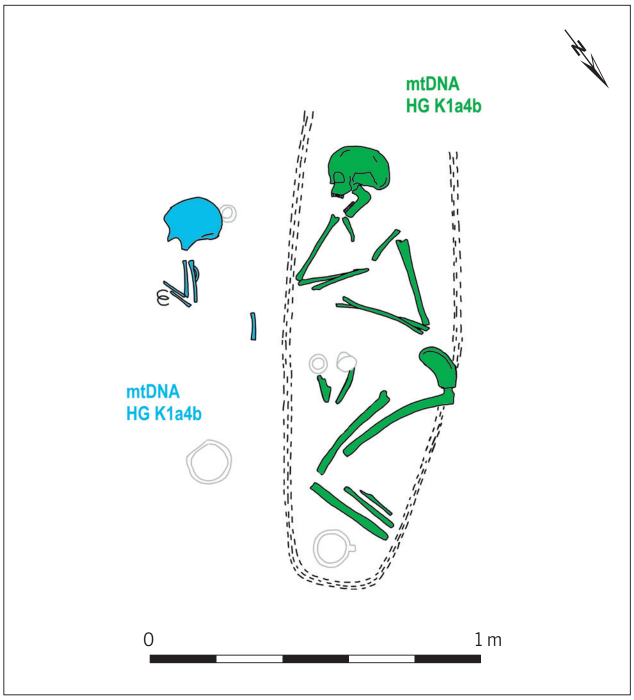

Fig. 5 Burials of a 14–16-year-old adolescent and 4-year-old child at Zwingendorf, Austria.

Abb. 5 *Bestattungen eines 14–16-jährigen Jugendlichen und eines 4-jährigen Kindes in Zwingendorf, Österreich.*

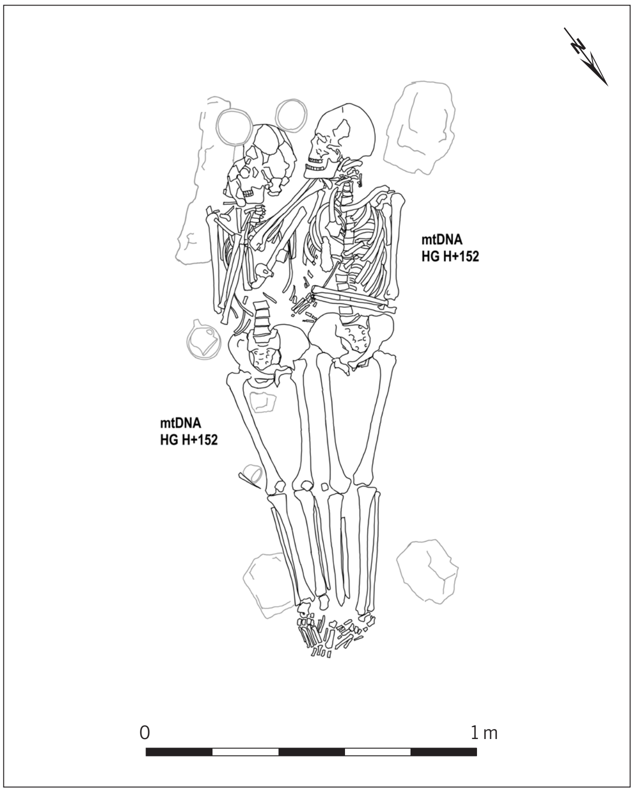

Fig. 6 Double burial of a 27–31-year-old and a 30–35-year-old male in close bodily contact at Schleinbach, Grave 30/31.

Abb. 6 *Doppelbestattung eines 27–31-jährigen und eines 30–35-jährigen Mannes in engem Körperkontakt in Schleinbach, Grab 30/31.*

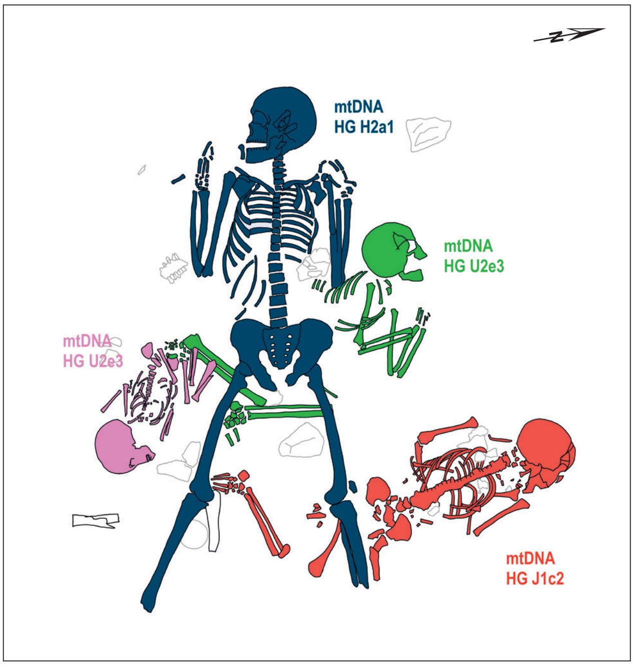

Fig. 7 Quadruple burial of a 30–35-year-old male, a 3–4-year-old girl, an 8–9-year-old boy and a 12-year-old girl from pit 60 at Schleinbach. No scale.

Abb. 7 *Vierfachbestattung eines 30–35-jährigen Mannes, eines 3–4-jährigen Mädchens, eines 8–9-jährigen Jungen und eines 12-jährigen Mädchens aus Grube 60 in Schleinbach. Ohne Maßstab.*

were found with, as the preservation did not suffice to recover their mtDNA profile.

The complete skeletons of a 2- and a 6-year-old child were found in storage pit 27. They were placed face to face in a firm embrace, which suggests a close emotional bond expressed through the burial position. The children in an embrace were found to share an H5a1 mitotype – they were maternally related and could have been siblings, although other types of relations, such as cousins and more distant relations, are also possible (Fig. 4).

Zwingendorf

Nine Early Bronze Age graves were excavated at Zwingendorf, Austria, in 1977 (Grefen-Peters 1982; Wewerka 1982). Although there are no radiocarbon dates available from Zwingendorf, typo-chronological dating places the site within the developed Únětice Culture at around 2000–1600 BC. The north-south oriented grave pits contained ten undisturbed individuals placed in flexed positions on the right sides of their bodies with heads to the south. Grave 8 contained the double burial of an adolescent aged between 14 and 16 years at death and a 4-year-old child. Although the individuals appear to share a grave, traces of a wood coffin were only documented around the adult. The child had been placed about 20 cm away; pottery was deposited at the feet and in the abdominal space of the adolescent and at the feet and head of the child. A bronze *Noppenring* was placed around the child's arm. The close positioning of the two individuals in Grave 8 suggests a personal relationship, even if the age difference between the individuals is probably not enough to suggest a mother-child relationship. MtDNA analysis confirmed a (close) maternal relationship between the adolescent and the child based on an identical K1a4b mitotype. A sibling/cousin relationship appears most likely (Fig. 5).

Schleinbach

Two small groups of graves and settlement features with more than 60 individuals were discovered at Schleinbach in the first half of the 20th century[4]. Grave 30/31 was found within a group of 18 graves arranged in two rows. Unusual for the Únětice context, two men were buried with the head in the south-west, in extended positions on their backs and very closely together; shoulders and legs were touching, whilst the arms and hands of the individual were folded over their upper bodies and partly overlapped (Fig. 6). The two male individuals, a 27–30-year-old and a 30–35-year-old, had identical fractures on the skull, probably inflicted by the same tool. Two ceramic bowls and two cups were found in association with the individuals. Their identical fate and shared treatment after death prompted an investigation of their genetic relationship. The individuals buried closely together in Grave 30/31 indeed share the same haplotype (H+152), suggesting a close maternal relationship.

The deposition of four individuals in a former storage pit at Schleinbach (Weninger 1954) included a 30–35-year-old male (60) and three children, an 8–9-year-old boy (60A), a 12-year-old girl (60B) and a 3–4-year-old girl (60C). The youngest girl had a perimortem impression fracture of the left parietal bone and was the only one placed according to the prevailing burial customs on the right side with the head towards the south-east. The boy was found on the left side of their body, oriented to the north-north-east, and the 12-year-old girl with the upper body in an extended position oriented to the north-east. The body of the adult male was deposited last, in an extended position with the head in the north-west, the upper arms next to the body, with elbows bent and hands adjacent to the shoulders, and legs slightly open (Fig. 7). The age gaps between the individuals and the haphazard mode of deposition suggest the eradication of a whole family.

MtDNA analysis revealed that the 8–9-year-old boy and 3–4-year-old girl from pit 60 share the same haplotype (U2e3a), whereas the 30–35-year-old male individual and 12-year-old girl belong to haplogroups H2a1 and J1c2. The male was not maternally related to the children but could have fathered the children with two different women.

Maternal relatedness

Within this study, we identified 18 unique mitochondrial haplogroups in 25 individuals, seven of which were represented in two individuals (Fig. 8). The haplogroups were estimated based on partial mitogenome sequences (see sequencing ranges in cf. Tab. 2), most of which represent almost full mitogenomes. Age estimates were determined according to Behar et al. (2012) and are listed (Tab. 3). All samples fall within modern European genetic variation and are also found at other archaeological sites. Haplogroup H, for example, is one of the most common mtDNA clades in Europe and is present in about 40 % of today's Europeans, and is thought to have resulted from demographic changes in the Middle Neolithic to Bell Beaker period (Brotherton et al. 2013). The sub-clade H2a1, found in the male individual from Schleinbach, Pit 60, has recently been found in three of 80 genetically typed individuals from the Lech River Valley in Bavaria (Knipper et al. 2017) and is present once in samples from Bronze Age Hungary (Allentoft et al. 2015). The subclade H2a1, as seen in the two children buried in an embrace from Unterhautzenthal 27, has also been identified in Bronze Age Estonia and Russia (Allentoft et al. 2015).

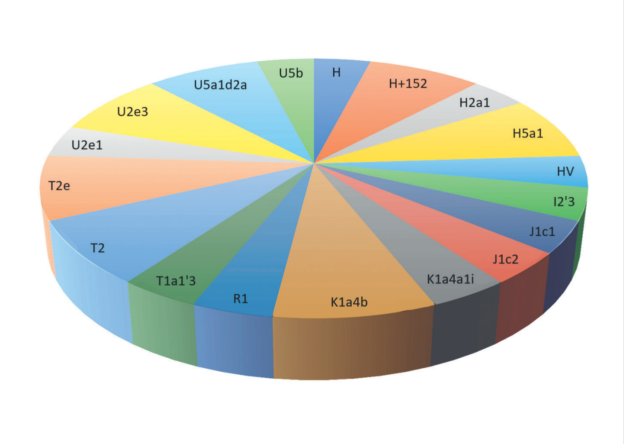

Fig. 8 Graphical representation of the 18 unique mitochondrial haplogroups in 25 individuals in this study.

Abb. 8 *Grafische Darstellung der 18 einzigartigen mitochondrialen Haplogruppen bei 25 Individuen in dieser Studie.*

4 Pany-Kucera et al. 2020; Rebay-Salisbury 2018a; Weninger 1954; Weninger 1954a

HG	Observations	Time Estimate [years]*	SD [years]*	
H	1	12846.0	773.4	
H+152	2	12846.0	773.4	
H2a1	1	7713.4	1754.3	
H5a1	2	6567.1	1533.8	
HV	1	21905.8	2832.7	
I2'3	1	11307.6	4153.7	
J1c1	1	10090.8	2228.7	
J1c2	1	9762.5	2010.7	
K1a4a1i	1	9617.5	2327.5	for K1a4a1
K1a4b	2	12051.5	3524.3	
R1	1	11420.5	3550.4	
T1a1'3	1	6997.3	2088.1	for T1a1
T2	2	19316.5	2545.1	
T2e	2	11858.8	3234.1	
U2e1	1	17873.5	3392.1	
U2e3	2	1047.3	4500.8	
U5a1d2a	2	3451.5	2709.3	
U5b	1	22794.0	3590.3	

* according to Behar et al. 2012

Tab. 3 Observed mtDNA haplogroups and time estimates (SD = standard deviation).

***Tab. 3** Beobachtete mtDNA-Haplogruppen und Zeitschätzungen (SD = Standardabweichung).*

In summary, we were able to provide mtDNA results for 11 archaeological contexts that included two or more individuals (Tab. 2) buried together at the same time or in close succession and referring to one another. The practice of burying two or more people together as part of the funerary ritual is not equally common during all prehistoric periods and in all areas. In Early Bronze Age Austria, double and multiple burials occur more often north of the Danube as part of the Únětice Culture than in areas that are culturally affiliated with Bavarian groups south of the Danube (Neugebauer 1994).

Our hypothesis of a close genetic relationship between co-buried individuals, based on the grave contexts, was corroborated by ancient mtDNA analysis in six out of 11 investigated archaeological contexts. For all but Franzhausen 860, we assume that the individuals lived contemporaneously and died in close succession. Taking the ages at death into account, we can propose the most likely types of kinship relations between individuals. In six contexts, we found identical haplotypes, which may suggest (close) maternal relatedness. The exact nature of relatedness, however, cannot be determined; mother/grandmother-child, aunt-niece/nephew, sibling and cousin relationships are possible, as well as more distant relationships.

Potential mother-child dyads were more difficult to trace than sibling/cousin relationships. At Franzhausen, the 35–50-year old female may have been the mother/grandmother or maternal aunt of the 7–8-year old child. We could reject the hypothesis that the 35–45-year-old female at Unterhautzenthal 95 was a maternal relative of the 3–4 and 4–5-year-old children, who were likely siblings. For three possible mother-child pairs, the mtDNA yield of the sub-adult was insufficient to investigate the relationship more closely.

Siblings or close maternal relatives like cousins via the maternal line were also found in Grave 30/31 from Schleinbach, in which two adult men with identical head traumata were buried, and in Pit 60 from the same site, which included four persons; two of the three children, the 3–4 and 8–9-year-olds, were related. The two children from Unterhautzenthal 27, 2 and 6 years old when they were buried embracing each other, also shared the mitochondrial haplotype.

Comparanda

How do our findings compare with other burial contexts of Late Neolithic/Bronze Age Europe? A study of the genetic relationship in four multiple burials of the Corded Ware complex (c. 2700–2200/2000 BC) from Eulau, Burgenlandkreis district, (Saxony-Anhalt, Germany) (Haak et al. 2008), revealed similar results. The burials followed a violent incident and the individuals were placed in meaningful positions to each other. Two graves contained four, one three and one two individuals. Grave 99 was that of a nuclear family, with a 40–60-year-old adult man and 35–50-year-old woman buried with children aged 4–5 and 8–9 at death; the woman shared the same mtDNA haplogroup with the children, and the boy shared the same Y chromosome haplogroup with the man. Grave 98, however, held the remains of a 30–38-year old woman who was excluded as being the biological mother of the three children in the grave (0–0.5–1, 4–5 and 7–9 years), two of which were found to share an mtDNA haplogroup. In the two remaining grave contexts, the DNA of the sub-adult individuals was not preserved well enough to prove further links between individuals.

The Bell Beaker cemeteries of Alburg and Irlbach, Straubing-Bogen disctrict (Bavaria in Southern Germany) (Sjögren et al. 2020), dated to c. 2750–2000 BC, comprising 24 and 18 single burials, respectively. The male individuals belong to a single Y-chromosome lineage, but again there is high vari- ability in the mtDNA, with 14 different mitochondrial haplotypes documented at Irlbach and nine at Alburg. Two pedigrees of four to six generations were linked primarily through the male line; and although there is strong evidence for fe- male exogamy, both through mtDNA diversity and isotope data, there are no direct matches between the mitochondrial haplotypes of the two communities located a mere 15 km apart. An exact match of the mitochondrial H5a1 haplotype was found in two individuals from Irlbach, a young child and a mature woman, but first- and second-degree relations could be excluded based on the full genome data.

Corded Ware (c. 2750–2460 BC), Bell Beaker (c. 2480–2150 BC) and Early Bronze Age (c. 2150–1700/1500 BC) individuals from several cemeteries in the Lech River Valley, Germany, have recently been subject to intensive interdisciplinary analysis, including kinship analysis using genome-wide data (Mittnik et al. 2019). Of 104 individuals, 39 first- and second-degree relationships were identified. Since single burials were the norm in this cultural context, related individuals were found in different graves, but in all but one case buried at the same site. Amongst the ten parent-offspring pairs, six were those of mothers and sons. Whereas the paternal lineage could be traced for four or five generations, with one exception, the maternal line lasted only one generation. The high diversity of mtDNA and the fact that mitochondrial haplotypes were shared almost exclusively between first-degree relatives (Mittnik et al. 2019) are important; this and the corroborating isotope evidence points to patrilocal residential rules. Non-local, high-status women have been identified at the burial sites, but curiously, none of their offspring; low-status individuals were found to be unrelated to the main families.

Of 312 excavated individuals from the Early Bronze Age cemetery of Mokrin, North-Banat disctrict (Serbia; 2700–1500 BC), 24 were tested and 15 were found to be involved in nine family relationships (Žegarac et al. 2021). These included three parent-offspring, two sibling and four second- and third-degree relationships. Related individuals were gen-erally buried close to one another. The population seemed genetically unstructured, with high diversity in both the Y-chromosome and mitochondrial haplogroups; 14 distinct mtDNA haplotypes were identified. The nine individuals with no genetic links to others were all female. Here too, female exogamy is suspected as a social mechanism underpinning the genetic findings.

A study of 80 mitochondrial genomes of Bronze Age individuals from Poland (c. 2400–1100 BC) found eight possible kinship connections in burial sites of the Trzciniec Cultural Circle, in which collective graves are common, as well as two pairs of possible maternal relatives at a Mierzanowice Culture site that include mostly single or double burials (Juras et al. 2020).

Of seven individuals deposited in a pit at Stillfried, Lower Austria in the Late Bronze Age c. 900–800 BC (Parson et al. 2018), a 45-year-old woman was found to share the established mitotype with the 5–6-year-old boy buried in close bodily contact. The archaeological context (Hellerschmid 2015) suggests the child was placed on her hip, with a second, 7–8-year-old boy next in the depositional sequence. A 30-year-old male, a three-year-old boy and a nine-year-old girl were also deposited in the pit; the other, a 40-year-old female of this context could be excluded as the mother of any of the children.

The published studies of Late Neolithic/Early Bronze Age kinship summarised above demonstrate both common traits and cultural variation. Bell Beaker, Corded Ware and Early Bronze Age communities all appear to be characterised by a high level of diversity in mitochondrial DNA but show differences in the way the population is otherwise structured. Genetic kinship could be more reliably traced through the male line, with individuals often buried in the vicinity of their relatives. This and supporting archaeological and isotopic evidence suggest a high level of female mobility, perhaps within super-regional marriage networks, but also for other reasons (Rebay-Salisbury 2023).

The increased use of vessels suitable for feeding infants in the Bronze and Early Iron Ages might indicate not only a change in childcare practices but also a change in mother-child relationships. Successful breastfeeding requires the mother and child to be physically connected most of the time, but with feeding vessels that contained milk from sheep, goats or cattle (Dunne et al. 2019), other members of the community could take over the task of feeding infants. This may have made mothering a more collective, shared responsibility within the community (Rebay-Salisbury et al. 2021).

Conclusion

Close personal relationships such as the mother-child bond were expressed through the way bodies were placed in relation to one another in the grave and frequently broadly based on kinship. Kinship is not the same as biological relatedness, even if there are overlaps, and we need to consider both the archaeological context information and the genetic data to understand how families were composed in the past. Double, multiple and consecutive graves (cf. Duday 2009) appear to be indicative of personal relationships, in particular when the placement of the bodies suggests love and care (Rebay-Salisbury 2018). What this means remains difficult to define – close bodily contact, embrace, interlocking limbs etc. may be part of how the burying community staged a close connection after death. It is perhaps easiest to define the expression of love and care in the burial practice by the clear contrast to its absence.

Not all burial evidence speaks of maternal love. On the contrary, evidence of abuse and neglect is frequent. A 3–4-year-old child from Unterhautzenthal 133, for example, had a broken humerus that was not corrected and healed in the wrong place. Nevertheless, the child was afforded a regular burial in the cemetery, and placed with care (Rebay-Salisbury et al. 2018b). At Schleinbach, a 5–6-year-old boy suffering from middle ear infections was disposed of in an Early Bronze Age storage pit. He died from no fewer than four blunt-force traumata to the skull, inflicted by at least two different kinds of objects or weapons (Rebay-Salisbury et al. 2020). Body parts of foetuses, infants and young children frequently appear in other graves, seemingly out of context, and most likely testify to the low social significance of some children within Central European Bronze Age communities.

In this study, we tested the genetic relationship between co-buried individuals through the maternal line by mtDNA analysis. Matches of mtDNA of individuals buried together in one grave are a strong argument that biological relatedness was a factor in kinship, a factor that was respected beyond death. However, several instances demonstrate that women appear staged in a mothering role in the burial context even when they were not directly biologically related. Ideologically at least, childcare was not exclusively associated with their biological mothers, which suggests that caregiving was likely organised communally, with the extended family – and perhaps entirely unrelated individuals – playing a significant role in bringing up children.

Acknowledgements

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1 base map © Google 2009
2 after Neugebauer/Neugebauer 1997, 287 Pl. 269
3 redrawn after Lauermann 1995, 33 Pl. 12; Lauermann 1996, 36 Fig. 17–19
4 redrawn after Lauermann 1991, 74 Fig. 22; and photos of the individuals on display in the MAMUZ Schloss Asparn/Zaya, Museum Mistelbach, Austria (taken by K. Rebay-Salisbury)
5 redrawn after Wewerka 1982, 29 Fig. 4
6 redrawn after Weninger 1954, 57 Fig. 4
7 redrawn after Weninger 1954a, 3
8 authors
Tab. 1 authors , NHM AA Inv. Nr. = inventory number of the Anthropological Department of the Natural History Museum in Vienna, Austria
Tab. 2 authors
Tab. 3 authors after Behar et al. 2012

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