

Female exogamy and gene pool diversification at the transition from the Final Neolithic to the Early Bronze Age in central Europe

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Human mobility has been vigorously debated as a key factor for the spread of bronze technology and profound changes in burial practices as well as material culture in central Europe at the transition from the Neolithic to the Bronze Age. However, the relevance of individual residential changes and their importance among specific age and sex groups are still poorly understood. Here, we present ancient DNA analysis, stable isotope data of oxygen, and radiogenic isotope ratios of strontium for 84 radiocarbon-dated skeletons from seven archaeological sites of the Late Neolithic Bell Beaker Complex and the Early Bronze Age from the Lech River valley in southern Bavaria, Germany. Complete mitochondrial genomes documented a diversification of maternal lineages over time. The isotope ratios disclosed the majority of the females to be nonlocal, while this is the case for only a few males and subadults. Most nonlocal females arrived in the study area as adults, but we do not detect their offspring among the sampled individuals. The striking patterns of patrilocality and female exogamy prevailed over at least 800 y between about 2500 and 1700 BC. The persisting residential rules and even a direct kinship relation across the transition from the Neolithic to the Bronze Age add to the archaeological evidence of continuing traditions from the Bell Beaker Complex to the Early Bronze Age. The results also attest to female mobility as a driving force for regional and supraregional communication and exchange at the dawn of the European metal ages.

mtDNA | strontium | oxygen | kinship | human mobility

Human mobility, ranging from individual residential changes to large-scale population replacements, was crucial for societal transformations. While for decades population change or stability was inferred from relics of material culture and burial customs, recent analytical developments have disclosed direct evidence from ancient human skeletal remains. Radiogenic and stable isotope analysis—particularly of strontium (Sr) and oxygen (O) bound in the hydroxyapatite of tooth enamel—has identified first-generation nonlocal individuals (1, 2), and ancient mitochondrial (3) and nuclear DNA data (4, 5) have revealed population continuity and abrupt or gradual replacements.

Archaeologists have vigorously debated population continuity and replacements during the transition from the Neolithic to the Bronze Age in central Europe in the late third millennium BC (6–8). South of the Danube, three archaeological cultural entities are commonly recognized: the Corded Ware Complex (CWC; 2800–2500/2200 BC), the Bell Beaker Complex (BBC; 2500–2150 BC), and the Early Bronze Age (EBA; 2150–1700/1650 BC). Genome-wide analyses demonstrated that burials of the central European CWC traced up to 75% of their ancestry to populations related to those living in the north Pontic steppes archaeologically classified as the Yamnaya Culture (4, 5). The exact reasons for the expansion

of steppe pastoralists to the west and east are currently unknown. Recent studies, however, suggest that a possible pandemic might have triggered this process by spreading a close relative of *Yersinia pestis*, the causative agent of bubonic plague (9, 10). On a smaller geographical scale, stable isotope data exhibited the crucial role of individual mobility, often connected with females (11, 12).

This paper focuses on the transition from the BBC to the EBA. Archeological evidence of the BBC includes burials with distinct bell-shaped ceramic cups, archery equipment, and occasionally gold, amber, and bronze objects (13, 14). Causes for its pan-European distribution from the Iberian Peninsula to the Carpathian Basin may have included human mobility, increasing trans-regional connectivity, or social differentiation accompanied by widely recognized symbols of prestige or status (15, 16). Consequently, the BBC was subject to one of the earliest large-scale Sr isotope studies (17, 18). It revealed multidirectional residential moves, which were related to subsistence practices rather than to actual mass migrations (19–21). In the subsequent EBA, human mobility was essential to distribute copper and tin ores, which do

Significance

Paleogenetic and isotope data from human remains shed new light on residential rules revealing patrilocality and high female mobility in European prehistory. We show the crucial role of this institution and its impact on the transformation of population compositions over several hundred years. Evidence for an epoch-transgressing maternal relationship between two individuals demonstrates long-debated population continuity from the central European Neolithic to the Bronze Age. We demonstrate that a simple notion of “migration” cannot explain the complex human mobility of third millennium BCE societies in Eurasia. On the contrary, it appears that part of what archaeologists understand as migration is the result of large-scale institutionalized and possibly sex- and age-related individual mobility.

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not occur together, as well as finished artifacts (22). Isotope analyses in EBA contexts revealed a heterogenic picture ranging from no clear indication for nonlocal individuals at Singen in southwest Germany (23) to a few nonlocal persons in mostly smaller burial communities in central Germany (24), while data from Únětice contexts in Silesia (Poland), were interpreted to be a reflection of “hypermobility” (25).

All this research illuminates the complex dynamics of social change and human mobility at the transition from the end of the Neolithic BBC to the EBA. To comprehend the mechanisms behind them it is crucial to investigate the spatial and quantitative scale of mobility as well as its relatedness to kinship, gender, and age. A suitable region to study this is the Lech River valley south of Augsburg (Bavaria, Germany) (Fig. 1 and *SI Appendix, Fig. S1*), where excavations over the last 20 y have unearthed almost 400 burials of both archaeological complexes and several associated EBA settlements, and radiocarbon dating of the

human remains revealed a chronological continuum from the BBC to the EBA (26).

Here we present genetic sex assignments and ancient mitochondrial DNA analyses as well as $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{18}\text{O}$ data for 84 human individuals from seven sites in the Lech River valley (Fig. 1 and *Dataset S1, Table 1*). The DNA analyses aimed at revealing direct maternal lineages connecting individuals from the same and different cemeteries, as well as between representatives of the BBC and EBA. The isotope analyses targeted the identification of first-generation nonlocals. The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in human tooth enamel depend on the geological conditions of the area from which foodstuffs were exploited during tooth formation in early life, while $\delta^{18}\text{O}$ values relate to temperature, elevation, and distance from the sea (1, 2) (*SI Appendix, Text S1*). All samples were recovered from a small area of ~10 km north–south and maximally 6 km east–west, which ensures well-comparable geologic and climatic conditions. The samples include 19 representatives of the BBC and 65 of the EBA of various age groups and both sexes. Fourteen faunal enamel samples helped to estimate the Sr isotope baseline.

Results and Discussion

Ancient DNA analysis.

Sex assignment. Of the 83 genetically studied individuals, 36 could be assigned as female and 34 as male, while assignment was not possible for the remaining 13 individuals (*Dataset S1, Table 2*). The genetic sex assignments agree with the archeological and—where possible—anthropological determinations in 62 cases and differ only in two cases. For six individuals, sex was only assigned genetically. Archeological sex assignments for BBC and EBA burials in this region consider sex-specific burial positions: women on the right side with their heads oriented to the south, and men on the left side with their heads oriented to the north (27). Sex is decisive for the burial position from early childhood onwards, as nine of the twelve infants and children that were genetically sexed were buried according to this custom.

mtDNA analysis. Complete or almost complete mtDNA sequences of at least 97% coverage could be reconstructed for 80 of the 83 samples. These fulfilled the criteria of ancient DNA authenticity as seen in increased deamination toward the DNA fragment ends (28) and a rate of contamination by modern human mtDNA estimated at below 5% (29) (*Dataset S1, Table 2*). We found 13 cases of haplotype sharing, which indicate a direct relationship through the maternal line. Most of these occurred within cemeteries, while in three cases the related burials were from different sites. Pairs of individuals with the same haplotypes were comprised of adult males, females, or subadults or included different combinations of sexes and age groups (*Dataset S1, Table 3*). In several cases, haplotype sharing occurred within double, multiple, or neighboring burials [Haunstetten, Unterer Talweg (UNTA) 58–62, feat. 150, 151, 152; Augsburg, Hugo-Eckener-Straße (HUGO) feat. 180; UNTA 58–62, feat. 67, 68 skeleton 2; and Wehringen-Hochfeld (WEHR) feat. 1415], pointing to a society where biological (maternal) kinship represented a significant form of social relationship that was honored in death (11). In addition, at the site of Wehringen-Hochfeld, three graves of individuals that shared an identical haplotype were the only ones to contain a large number of Bronze grave goods (*SI Appendix, Fig. S2*), indicating that social status and/or material wealth could be transferred by maternal kinship.

The most important finding from an archaeological perspective is the shared haplotype between the BBC individual WEHR 1192 skeleton A (2331–2150 cal BC with 95.4% probability) and the EBA individual Königsbrunn-Obere Kreuzstraße (OBKR) feat. 93 (2111–1907 cal BC with 95.4% probability). Based on radiocarbon dates, they lived between 0 and 10 generations apart, which suggests a common maternal ancestor and local genetic continuity in a maternal lineage spanning the transition from the Neolithic to the Bronze Age.

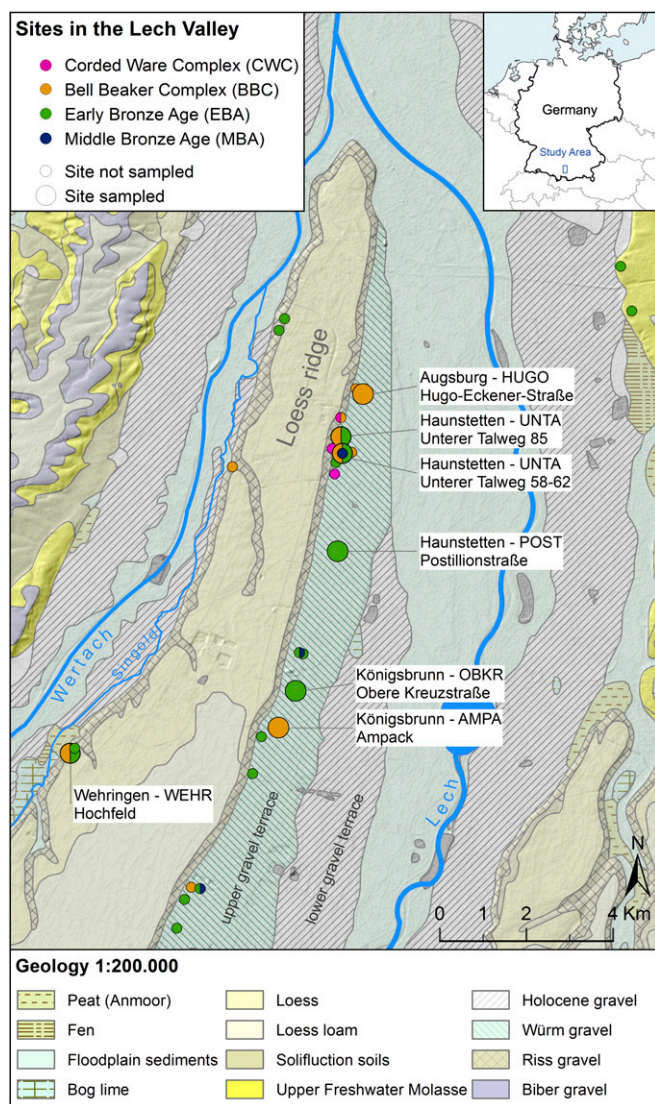


Fig. 1. Investigated BBC and EBA cemeteries in the Lech River valley. All sites are located along a loess ridge and share similar environmental conditions. Produced using Copernicus data and information funded by the European Union—EU-DEM layers; Geology 1:200.000. Data copyright Bundesanstalt für Geowissenschaften und Rohstoffe (BGR). (Graphic: S. E. Metz.)

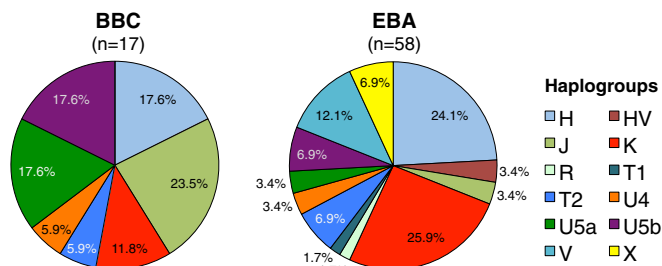


Fig. 2. Pie charts of mtDNA haplogroups of BBC and EBA individuals. Identical haplotypes in multiple graves or neighboring burials are counted only once. (Graphic: A. Mitnik.)

Overall, we found 61 unique haplotypes among the 80 genetically typed individuals. This considerable diversity seen in maternal lineages ($H_d = 0.9924 \pm 0.0004$) could be explained by an exogamous social organization as has been proposed previously for Final Neolithic societies (11). We also see an apparent diversification of maternal lineages in the EBA with additional haplogroups absent among the BBC burials (HV, R, T1, V, and X; Fig. 2 and Dataset S1, Tables 2 and 4). However, this is not necessarily due to increased gene flow into the population, as a test of population continuity based on haplogroup frequencies (3) does not reject the null hypothesis of frequency changes due to simple genetic drift in a continuous local population, even when assuming only one generation separation between the BBC and EBA individuals ($P = 0.067$).

A genetic distance analysis (fixation index F_{st}) of the mtDNA hypervariable segment-I (HVS-I) region including other Neolithic and Early Bronze Age groups from the more northerly Mittelelbe–Saale region (3) shows no significant differentiation between both Lech River valley populations ($F_{st} = 0.03725$, $P = 0.117$; Dataset S1, Table 5), again supporting population continuity. However, a lack of significant genetic distance between the BBC group and other contemporaneous groups might also be due

to its comparatively low sample size. In contrast, the EBA population of the Lech River valley shows greater genetic distance from other contemporaneous European populations, with significant results seen in comparison with both the BBC and the EBA Únětice populations from the Mittelelbe–Saale region (Dataset S1, Table 5).

Stable Isotope Analysis.

Strontium isotope data. Sr and O isotope analysis concentrated on second permanent molars of 83 individuals and used alternative teeth in case of their absence. In a second stage, we determined $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for third molars and one first molar of those individuals who appeared nonlocal in their first samples. The $^{87}\text{Sr}/^{86}\text{Sr}$ values of all human samples varied between 0.70820 and 0.71505 (Dataset S1, Table 2 and Fig. 3). Based on one sample per individual and using the earlier forming tooth of tooth pairs, there is no statistically significant difference among the isotope ratios found for the different burial communities [one-way ANOVA; $F(5, 77) = 0.7773$, $P = 0.5691$]. This agrees with the prevalence of Würm and Riss gravel along the loess ridge in the near vicinity of the sites. Only at Wehringen $^{87}\text{Sr}/^{86}\text{Sr}$ values of between 0.7080 and 0.7090 did not occur (SI Appendix, Fig. S3), suggesting small-scale differences of the baseline values and largely localized land use patterns.

Most striking was the significant difference between the data spectra of the males and subadults on the one hand and the females on the other [one-way ANOVA: $F(2, 79) = 9.0885$, $P = 0.0003$; one tooth per individual; Fig. 3]. The subadult individuals yielded $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of between 0.70832 and 0.71025 (average: 0.70939 ± 0.00052 ; 1 SD; $n = 26$), excluding an outlier at the 4-sigma level with $^{87}\text{Sr}/^{86}\text{Sr} = 0.71301$ (UNTA 148). The Sr isotope ratios of the males, using the only analyzed or the earlier forming tooth of tooth pairs, varied between 0.70820 and 0.71046 (average: 0.70938 ± 0.00060 ; 1 SD; $n = 26$), excluding an outlier with $^{87}\text{Sr}/^{86}\text{Sr} = 0.71505$ (UNTA 68 SK 1). The difference between the males and the subadults was statistically insignificant [Student's t test: $t(52) = -0.2563$, $P = 0.7987$]. The data spectrum of the females ranged

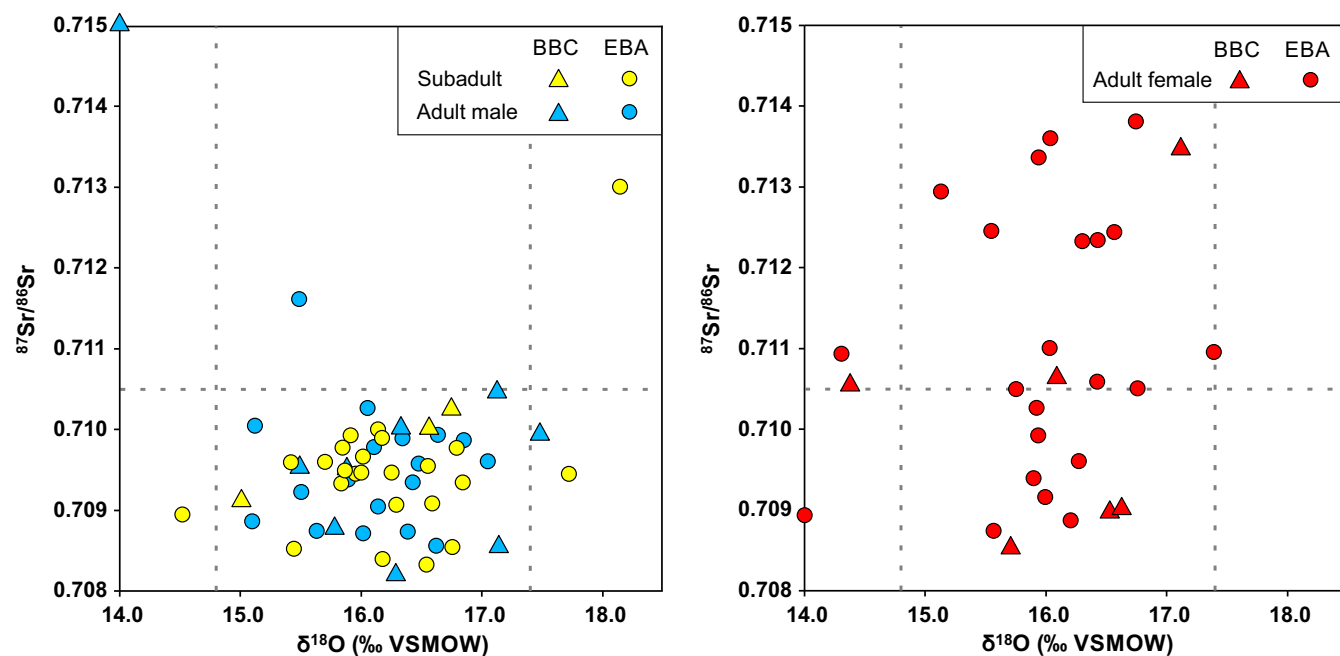


Fig. 3. Scatter plot of $\delta^{18}\text{O}_p$ and $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of subadult and adult male individuals (Left) and adult female individuals (Right) from the Lech River valley. The hatched lines indicate the local ranges for both isotope ratios (for comparison, see SI Appendix, Figs. S4 and S5). Samples plotted on the y axis lack $\delta^{18}\text{O}_p$ data. VSMOW, Vienna standard mean ocean water. (Graphic: C. Knipper.)

from 0.70854 to 0.71381, with a higher average and larger SD than those of the subadults and the males (average 0.71085 ± 0.00168 ; 1 SD; $n = 28$). The differences between the females and the subadults [Student's t test: $t(40.67) = -3.7306$, $P = 0.0006$] and between the females and the males [Student's t test: $t(53) = -3.1597$, $P = 0.0026$] were statistically significant.

The dissimilarity between the sexes was due to a larger number of females exhibiting $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of 0.71050 and above ($n = 17/28$) than were found among the males ($n = 1/26$ or $2/26$ if both teeth of tooth pairs are considered) and subadults ($n = 1/26$). The cutoff at 0.71050 for the identification of nonlocal individuals is based on archeological faunal enamel analyzed in this study and literature data from the Lech River valley (18, 30). Of 14 enamel samples of contemporaneous small wild (dormouse or squirrel) and domestic animals (pig, sheep/goat, cattle), 12 revealed $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of between 0.70805 and 0.7102, while a cattle tooth and a pig tooth yielded more radiogenic values of 0.71166 and 0.71455, respectively (SI Appendix, Fig. S4 and Dataset S1, Table 6). Archeological human (18) and animal bones (30) as well as human cremated bones (30) from the Lech River valley confirm this range. Moreover, the northern Pre-Alpine lowlands have been subject to numerous Sr isotope studies, including a mapping project (30). With more than 1,000 biological samples, the area shown in SI Appendix, Fig. S1 is one of the best-studied worldwide (Dataset S1, Table 7). At sites on Pleistocene loess and gravel, 379 of 474 (80%) human enamel, 177 of 179 (99%) human bone, 74 of 88 (84%) animal bone, 6 of 6 (100%) animal enamel, and 99 of 101 (98%) human cremated bone samples yielded $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of below 0.7105, while more radiogenic values dominate in neighboring regions, such as the Nördlinger Ries, the Bavarian Forest, and the inner Alps.

For 18 individuals, we sampled tooth pairs of mostly second (M2) and third molars (M3) (Dataset S1, Table 2 and Fig. 4). In 11 of 14 females, the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the M3s resembled those of the M2s closely with differences of between 0.00010 and 0.00049 and ratios of above 0.71050 in both teeth. This implies that these non-local females moved to the Lech River valley after their tooth crown

formation was complete, i.e., older than 16 y of age (31). The individuals POST 99, with both ratios above the local range, but a larger difference of 0.00126, and UNTA 110, with a small difference of 0.00034, but the M2 above and the M3 below the cutoff of 0.71050, likely also belonged to this group. In contrast, an M2 of OBKR 26 had an $^{87}\text{Sr}/^{86}\text{Sr}$ ratio above the local range, while the ratio in an M3 was lower by 0.00146 and fell into the local range. This indicates movement during childhood. The male UNTA 153 exhibited an $^{87}\text{Sr}/^{86}\text{Sr}$ ratio within the local range in his M1 and a more radiogenic M3. The data suggest an origin in the Lech River valley or another loess landscape with similar baseline values, movement into a geologically different area during childhood, and a return or movement to the Lech River valley in adulthood.

Oxygen isotope data. The O isotope ratios of the phosphate group of enamel bioapatite ($\delta^{18}\text{O}_\text{p}$) ranged between 14.3 and 18.2‰ (average $16.2 \pm 0.7\text{‰}$; $n = 82$). An exclusion of first molars and deciduous teeth with potentially elevated values due to breastfeeding (32) revealed a slightly lower average of $16.1 \pm 0.7\text{‰}$ ($n = 65$). There were no statistically significant differences among the sites [one-way ANOVA: $F(5,76) = 0.5643$, $P = 0.2967$] or among the subadult and adult individuals of both sexes [one-way ANOVA: $F(2,77) = 0.5679$, $P = 0.5691$]. Overall, the data are well comparable with earlier studies in southern Germany (12, 20, 23, 33) (SI Appendix, Fig. S5). Modern precipitation is also a proxy for regionally typical values when converted into $\delta^{18}\text{O}_\text{p}$ using linear regressions (34–36). Combining weighted annual average data at southern German monitoring stations (37) (converted with equation 6 in ref. 35) and the Lech River valley human dataset itself (average excluding deciduous teeth and first molars ± 2 SD) suggests that regionally typical $\delta^{18}\text{O}_\text{p}$ values ranged between about 14.7 and 17.4‰ (SI Appendix, Fig. S5). Compared with this range, the individuals UNTA 148, WEHR 1415B, HUGO 180/1, and OBKR 73 exhibited higher values, while the samples OBKR 9A, OBKR 9B, and HUGO 168 had lower values. Exclusive considerations of other equations (34 and 36) seemed to overestimate the number of nonlocal individuals, even though influence of short-term climate alterations, seasonality, and food preparation also needs to be considered (2, 38).

Among the individuals whose $\delta^{18}\text{O}_\text{p}$ values appeared nonlocal, UNTA 148, OBKR 9A, HUGO 168, and OBKR 73 also had $^{87}\text{Sr}/^{86}\text{Sr}$ ratios above the local range. The elevated $\delta^{18}\text{O}_\text{p}$ value of a deciduous tooth of WEHR 1415B may be due to breastfeeding rather than a nonlocal origin. This leaves OBKR 9B (a 6- to 8-y-old girl) and the adult male HUGO 180/Ind. 1 to be identified as nonlocal solely based on their O isotope compositions.

Patrilocal residential rules, kinship ties, female exogamy, and supraregional connections. The combination of Sr and O isotope data identified 22 of 83 investigated individuals (26.5%) as nonlocal or temporarily living away from the Lech River valley during childhood. Among them were 17/28 (60.7%) females, 3/27 (11.1%) males, 2/27 (7.4%) subadults, and 0/1 (0%) adult individuals of indeterminate sex (Dataset S1, Table 8). These individuals were distributed equally among the sites and burials of the BBC (5/18 = 27.8%) and the EBA (17/65 = 26.2%). The numbers are minimum estimates because the local isotope ranges are not exclusive to the Lech River valley, and individuals with similar $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of both sampled teeth may have moved undetectably between geologically similar landscapes.

The high proportion of nonlocal females occurs at the same time as a high and increasing haplotype diversity, i.e., a high diversity of maternal lineages, and is consistent with a patrilocal residential system and female exogamy (39, 40). Predominantly nonlocal females were also found in BBC contexts in southern Bavaria (especially if 0.71050 is used as $^{87}\text{Sr}/^{86}\text{Sr}$ cutoff), Austria, Hungary, the Czech Republic (18), and Britain (21), among CWC burials in southern Germany (12), and EBA inhumations in central Germany (24), pointing to a supraregional prevalence of patrilocal residential rules. The pattern is, however, most striking in the Lech River valley.

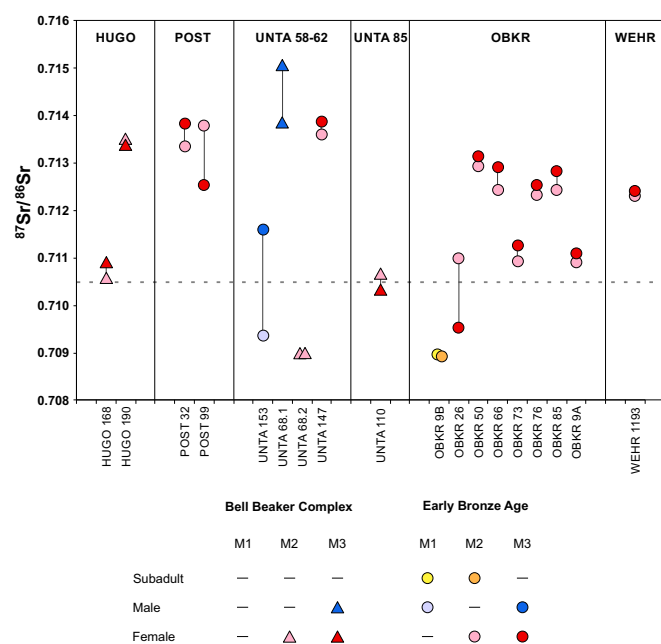


Fig. 4. Pairs of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of early- and late-forming teeth from the same individuals. The hatched line indicates the upper limit of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the locally biologically available strontium (for comparison, see SI Appendix, Fig. S4). (Graphic: C. Knipper.)

Here, the Sr isotope data of the nonlocal females tended to cluster in two groups—one between about 0.7105 and 0.7110 and the other between 0.71233 and 0.71381. This suggests different regions of origin, although specific assignments are problematic. The nearest occurrence of more radiogenic Sr and contemporaneous archaeological sites is in the Nördlinger Ries (41) about 60 km away (*SI Appendix, Fig. S1*). More distant possible areas of origin include the inner Alps (30), the Bavarian, Bohemian, and Black forests (42), and sediments of the Saale Glaciation in central Germany (24). Among them, the Bavarian and the Black forests have only sparse archaeological records and are therefore rather unlikely, while Bohemia and central Germany revealed strong evidence of contemporary settlement. The Lech River valley belonged to a supraregional phenomenon during the BBC (43) and participated during the EBA in long-distance communication networks (20). However, the isotopically identified nonlocal females are not archaeologically conspicuous. They exhibited the posture or orientation typical for southern Germany and were neither isolated nor concentrated in certain areas of the cemeteries. Eight of the EBA females—both local and nonlocal—also wore a particular type of elaborate head-dress, which was common in southern Bavaria. Neither nonlocal nor local individuals had a common ensemble of grave goods.

The arrival of the females in adolescence or later suggests the establishment of partnerships following patrilocality rules as a possible reason for their movements (44), while the archaeological evidence points to their full integration into the respective communities. Identical mtDNA haplotypes only occurred among isotopically local individuals except for the nonlocal male HUGO 180/Ind. 1 (Fig. 5). This argues for local settlement continuity spanning multiple generations and underlines the nonlocal origins of the females with the exotic Sr isotope ratios. Strikingly, we failed to identify offspring of the nonlocal females, as none of the subadults or adults of either sex shared an identical haplotype with any of them. Besides sample selection and preservation, conceivable causes

include—possibly culturally triggered—unsuccessful reproduction, or even movement of the offspring away from the Lech River valley.

Conclusion

Interdisciplinary research combining archaeological, Sr and O isotope, and mtDNA analyses disclosed striking evidence for patrilocality rules and female exogamy persisting over at least 800 y in central Europe from about 2500 to 1700 BC, and possibly starting even earlier in the CWC (12). On the one hand, the archaeological record, radiocarbon dates, burial practices, mtDNA haplogroups, and isotope data—especially of male and subadult individuals—support settlement continuity from the Late Neolithic BBC to the EBA. This includes indication of direct maternal kinship between a male with BBC and a female with EBA attributes. On the other hand, numerous nonlocal Sr isotope ratios among the females and the diversification of the maternal lineages point to exogamy and female mobility as a driving force of regional and supraregional communication and knowledge transmission in a time of major innovations. Systematic individual movements are an important factor in third millennium BCE societies in Eurasia and force us to reexamine evidence of “migration” that may actually be the result of large-scale institutionalized and possibly sex- and age-related individual mobility.

Materials and Methods

Archaeological Background. Since the 1980s, excavations in the Lech River valley south of the city of Augsburg (Bavaria, Germany; Fig. 1 and *SI Appendix, Fig. S1*) have unearthed cemeteries of the Late Neolithic BBC and the Early and Middle Bronze Age, some of them covering multiple periods. Some of the EBA sites are associated with a hamlet. Cultural attribution is based on archaeological criteria such as grave goods. Direct radiocarbon dating of the human remains revealed age ranges of 2500–2150 cal BC for the BBC burials and 2150–1650/1600 cal BC for the EBA burials (26). Eighty-four sampled individuals derive from seven cemeteries and include all burials of combined BBC and EBA sites and a selection from larger EBA cemeteries based on chronologically significant or remarkable grave goods and a representative number of children and adults of both sexes (*Dataset S1, Tables 1 and 2*).

Ancient DNA Analysis. Teeth were sampled in clean room facilities at the University of Tübingen by sawing them apart transversally at the border of crown and root and removing dentine from inside the crown with a sterile dentistry drill. Extraction was performed following an established protocol (45), and an aliquot of extract was used to generate double-indexed libraries (46, 47) that were sequenced before and after enrichment for mtDNA (48) on a NextSeq with $2 \times 101+8+8$ and a HiSeq2500 with $2 \times 101+8+8$ cycles. Negative controls were included in the extraction and library preparation and taken along for all further processing steps (*Dataset S1, Table 9*). Base call files produced by the instrument's software were converted to raw sequences that were demultiplexed according to the index combinations they received during library preparation. A software pipeline was used (49) to clip adapter sequences, map reads to the reference—hg19 for shotgun data and revised Cambridge reference sequence (rCRS) for mitochondrial capture data—using Burrows–Wheeler Aligner (BWA) (50) with parameters “-l 10000 -n 0.01,” and to remove duplicate reads. Mitochondrial consensus sequences were called while jointly estimating the rate of deamination damage and contamination from present-day human sources with the probabilistic iterative method applied in the software *schmutzi* (29) using the accompanying tool *log2fasta* with parameter *-q* 20. Poly-C regions and mutational hotspots at positions 303–315, 515–522, and 16519 were masked.

Sex was assigned on the basis of the shotgun-sequencing data (51), restricted to reads that showed postmortem damage with the script *pmdtools* (52), parameter “-threshold 3.” Mitochondrial haplogroups were assigned manually by consulting the output of Haplofind (53) and Haplogrep2.0 (54). Haplogroup frequencies and F_{st} analysis were conducted on a conservative dataset, counting only once a shared haplotype that occurred within a multiple burial or between very closely located burials. Pairwise F_{st} comparisons against other ancient populations (3) were performed with Arlequin ver3.5.2 (55) using the Tamura & Nei substitution model and gamma value 0.300 on the HVS-I region (16126–16369), excluding the poly-C-stretch at 16184–16195. The significance level was corrected for using the Benjamini–Hochberg correction to limit false positives (56). To assess if the different haplogroup frequencies seen in BBC and EBA can be attributed to genetic drift alone, the Test for Population Continuity (3) was applied. All mtDNA sequences generated in this study are deposited in GenBank (accession numbers: MF498658–MF498737).

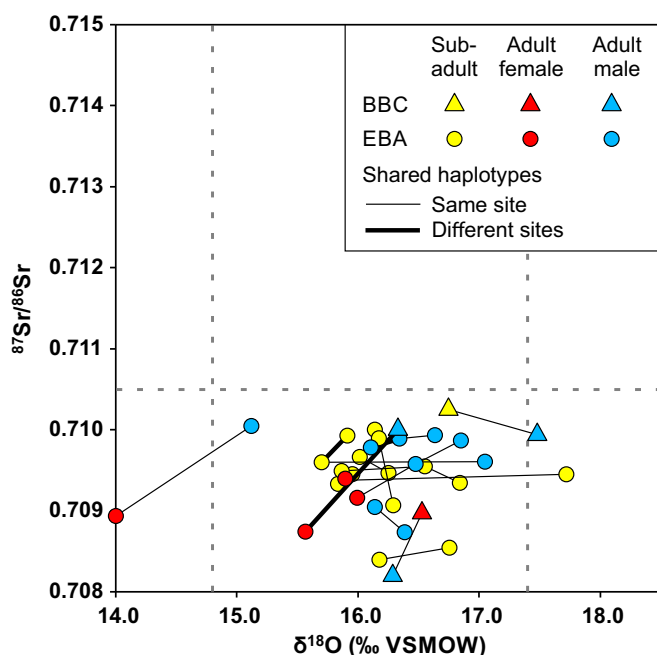


Fig. 5. Scatter plot of $\delta^{18}\text{O}_p$ and $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of individuals with shared haplotypes. Thin lines connect individuals from the same site. Fat lines connect individuals from different sites. The hatched lines indicate the local ranges for both isotope ratios (for comparison, see *SI Appendix, Figs. S4 and S5*). (Graphic: C. Knipper.)

Strontium and Oxygen Isotope Analyses. Sr and O isotope analyses on enamel samples were carried out at the Curt Engelhorn Centre for Archaeometry in Mannheim and the Department for Applied and Analytical Paleontology at the University of Mainz, Germany, following previously described methods (57, 58) (for comparison, see [SI Appendix, Text S1](#)). Statistical analysis was carried out using WinSTAT for Excel 2007.1 and Sigma Plot. All datasets used in Student's *t* tests were tested for normality using the Kolmogorov–Smirnov test.

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