Ancient genome-wide analyses infer kinship structure in an Early Medieval Alemannic graveyard

Niall O’Sullivan 1,2,3*, Cosimo Posth 2,4, Valentina Coia 1, Verena J. Schuenemann 4,5†, T. Douglas Price 6, Joachim Wahl 7,8, Ron Pinhasi 3,9, Albert Zink 1*, Johannes Krause 2,4*, Frank Maixner 1*

From historical and archeological records, it is posited that the European medieval household was a combination of close relatives and recruits. However, this kinship structure has not yet been directly tested at a genomic level on medieval burials. The early 7th-century CE burial at Niederstotzingen, discovered in 1962, is the most complete and richest example of Alemannic funerary practice in Germany. Excavations found 13 individuals who were buried with an array of inscribed bridle gear, jewelry, armor, and swords. These artifacts support the view that the individuals had contact with France, northern Italy, and Byzantium. This study analyzed genome-wide sequences recovered from the remains, in tandem with analysis of the archeological context, to reconstruct kinship and the extent of outside contact. Eleven individuals had sufficient DNA preservation to genetically sex them as male and identify nine unique mitochondrial haplotypes and two distinct Y chromosome lineages. Genome-wide analyses were performed on eight individuals to estimate genetic affiliation to modern western Eurasians and genetic kinship at the burial. Five individuals were direct relatives. Three other individuals were not detectably related; two of these showed genomic affinity to southern Europeans. The genetic makeup of the individuals shares no observable pattern with their orientation in the burial or the cultural association of their grave goods, with the five related individuals buried with grave goods associated with three diverse cultural origins. These findings support the idea that not only were kinship and fellowship held in equal regard: Diverse cultural appropriation was practiced among closely related individuals as well.

INTRODUCTION

The Alemanni were a confederation of Germanic tribes that inhabited the eastern Upper Rhine basin and surrounding region (Fig. 1). Roman ethnographers mentioned the Alemanni, but historical records from the 3rd to the 6th century CE contain no regular description of these tribes (2). The upheaval that occurred during the European Migration Period (Völkerwanderung) partly explains the interchangeability of nomenclature with the contemporaneous Suebi people of the same region and periods of geographic discontinuity in the historical record (3). This diverse nomenclature reflects centuries of interactions between Romans and other Germanic groups such as the Franks, Burgundians, Thuringians, Saxons, and Bavarians. With the defeat of the Alemanni by Clovis I of the Franks in 497 CE, Alamanni became a subsumed Duchy of the Merovingian Kingdom. This event solidified the naming of the inhabitants of this region as Alemanni (3). From the 5th to the 8th century CE, integration between the Franks and the Alemanni was reflected by changed burial practices, with households (familia) buried in richly furnished graves (Adelsgräber) (4). The splendor of these Adelsgräber served to demonstrate the kinship structure, wealth, and status of the familia and also the power of the Franks (Personenverbandsstaaten, a system of power based on personal relations rather than fixed territory). Because inclusion in familia during the Merovingian period was not necessarily based on inheritance or provenance, debate continues on the symbolism of these burial rites (5).

The 7th-century CE Alemannic burial site at Niederstotzingen in southern Germany, used circa 580 to 630 CE, represents the best-preserved example of such an Alemannic Adelsgräber. Discovered in 1962 (Fig. 1) (6, 7), 13 human skeletal remains (10 adults and 3 infants) were excavated from 12 graves. The grave goods indicated contacts with Byzantines, Lombards, and Franks (Table 1 and fig. S1). These outside contacts appear to have been facilitated by equestrianism and guardianship of a nearby Roman crossroads. Two of the 12 graves (3 and 12) were multiple burials containing three individuals each, from which it has been inferred that they had close familial relationships (8). Both multiple burials contained Byzantine artifacts, lamella helmet (grave 12, individual 12B), and equestrian gear with Byzantine engravings (grave 3, individual 3A), suggesting eastern Mediterranean contact, whereas those buried with Frankish (individual 9) and Lombard (individual 6) artifacts support contact with eastern France and northern Italy (6, 8, 9).

Since the initial discovery of Niederstotzingen, bioarchaeological analyses have provided additional information from the remains (section S2). Strontium and oxygen isotope data from the enamel showed that most individuals are local rather than migrants (Table 1, table S2, and fig. S2), except for individuals 10 and 3B. Polymerase chain reaction (PCR)–based sex estimation and reconstruction of the mitochondrial DNA (mtDNA) hypervariable region offered the first genetic characterization of the individuals (table S1) (8). Despite these findings, there are still questions regarding...
their genetic sex, kinship, and genetic origin, because of the technical challenges of ancient DNA (aDNA) analysis (10). Typically, DNA extracted from archeological remains contains only trace amounts of endogenous human DNA. Furthermore, multiple waves of migration and recent admixture can make it challenging to genetically distinguish ancient populations inhabiting nearby geographical locations (11, 12). Single-nucleotide polymorphisms (SNP) in recombining regions of autosomes and the X chromosome are especially...
useful for analysis of population affiliations (13, 14) and kinship (15, 16). These SNPs contain ancestral information from a large number of an individual’s ancestors, unlike the nonrecombinant maternally inherited mtDNA and paternally inherited Y chromosome each representing only an ancestral lineage.

To increase the resolution of kinship and population genetic analysis, we analyzed the individuals recovered from Niederstotzingen by using an approach that combined targeted DNA enrichment of more than 1 million SNPs (17) with high-throughput sequencing. Through joint analysis of mtDNA, Y chromosome [the nonrecombinating region (NRY)], and autosomal SNPs, we aimed to reconstruct potential familial relationships of the Niederstotzingen individuals and estimate their genetic sex. In combination with archeological and isotopic data, we directly tested historical and archeological hypotheses positing that Alemannic burial practices, through assortment of deceased individuals and their associated artifacts, are a reflection of mobility and kinship structure. Our results provide insight into the medieval household (familia) and the role of multiple burials at Niederstotzingen. Previous studies have shown that the genetic affiliations of ancient people often do not match putative geographic origin of their culture (5, 18). Hence, we investigated the congruence between the biological provenance and the cultural origin of burial rites and goods present at Niederstotzingen. Our results shed new light on the kinship structure in Early Medieval Europe and investigate whether contacts at Niederstotzingen to southern Europe and elsewhere went beyond the exchange of material artifacts. The results show that 11 of the individuals are likely males, suggesting a sex-biased burial practice, 5 of whom are detectably related to at least second degree. These five related individuals had culturally diverse grave goods despite the evidence that all of them showed local isotope signals with northern European genetic affiliations; these data show how diverse cultural appropriation could exist even among close relatives.

RESULTS

Authentication of sequenced data

To assess the quantity and quality of the extracted DNA, we performed shotgun sequencing before capture and mapped the sequences to the human genome. The percentage of endogenous DNA varied considerably between sequenced samples (<1 to 75%), showing different rates of endogenous DNA preservation among individuals and even in subsamplings from the same individual (table S3). Captured libraries showed increased percentage abundance mapping at target loci compared to shotgun libraries: between 273- and 2494-fold target enrichment efficiency for mtDNA and between 22- and 232-fold target enrichment efficiency for the 1240K SNP capture (tables S4 and S5). Deamination patterns at the 5′ and 3′ ends, typical of aDNA damage in non-UDG (uracil-DNA glycosylase)–treated libraries (fig. S3), indicate that there are endogenous sequences present. Low contamination estimates of the uniparental markers obtained with the software Schmutzi on mtDNA and ANGSD (analysis of next-generation sequencing data) on the X chromosome further support the authenticity of our ancient human DNA (Table 1 and tables S6 and S7). Exceptions were individuals 2, 10, and 12A that had a median mtDNA contamination above 5%. For these mitogenomes, low-covered and ambiguous mtDNA positions were further stringently filtered through visual inspection with Integrative Genomics Viewer (19) and compared with PMDtools filtered reads.

Analysis of uniparental markers

mtDNA haplogroups were successfully assigned to all 13 individuals (Table 1). Notably, there are three groups of individuals that share, among the assigned positions, identical haplotypes: individuals 4, 9, and 12B in haplogroup X2b4; individuals 1 and 3A in haplogroup K1a; and individuals 2 and 5 in haplogroup K1a1b2a1 (table S8). Postmortem deamination (PMD) PMDtools filtering (threshold 3) of mtcapture sequences of individuals 2, 10, and 12A (that showed contamination estimates greater than 5%) did not change the main haplogroup; only 12A’s haplogroup becomes less derived (table S14).

Despite low coverage across the Y chromosome in the 1240K, the NRY haplogroups of 10 individuals were successfully recovered (Table 1 and tables S3 and S9). Most individuals belong to the R1b haplogroup (individuals 1, 3A, 3C, 6, 9, 12A, 12B, and 12C), which has the highest frequency (>70%) in modern western European populations (20). Five individuals (1, 3A, 9, 12B, and 12C) share the same marker (Z319) defining haplogroup R1b1a2a1a1c2b2b1a1. Because of incomplete SNP capture and coverage on the Y chromosome, most of the individual’s haplotypes do not overlap across the entire International Society of Genetic Genealogy (ISOGG) Y-haplogroup tree, but multiple positions with the same haplotype and/or consistent root allow estimation of Y chromosome ancestry anyway. For example, individuals 1, 3A, and 6 have R1b lineage and marker Z347 (R1b1a2a1a1c2b2b), which belongs to the same male ancestral lineage as marker Z319. Individual 3B instead carries NRY haplogroup G2a2b1, which is rare in modern north, west, and east European populations (<5%), only reaching common abundance in the Caucasus (>70%), southern Europe, and the Near East (10 to 15%) (21).

Sex determination of individuals

Genetic-based sex estimates, using shotgun and 1240K capture data, show that at least 11 of the individuals were probably male. From shotgun sequences, 1, 3A, 3B, 3C, 4, 6, 9, 12B, and 12C have statistically significant rates of male DNA, while 2, 5, 10, and 12A are low-covered but consistent with the presence of male DNA (Table 1 and table S10). To further validate authenticity of these data, shotgun sequences were filtered for reads with PMD with PMDtools (threshold 3) (22). Sex estimates were largely consistent for filtered data, except 3A, 3C, and 12B, which lost statistical significance, and individuals 2 and 5, which had insufficient reads remaining (table S13). The normalized ratio of X chromosome to autosomal reads provided a robust estimate of sex for shotgun libraries that had extremely low coverage, except for individuals 5 and 12a (table S11) (23). The ratio of captured X and Y chromosome SNPs to autosomal SNPs in the 1240K also shows a similar proportion of X and Y SNPs, further supporting the indication that all the individuals are males (fig. S4 and table S10) (24).

Genotyping and population affinity of genome-wide capture

After 1240K capture, between 29,673 and 374,347 called SNPs overlapped with the Human Origins Database (25, 26), providing enough genomic markers for accurate estimates of genetic affinity (1, 3A, 3B, 3C, 6, 9, 12B, and 12C) (Table 1 and table S5). The projections of the ancient genomic data on principal components analysis (PCA) built with modern west Eurasians show that six individuals from Niederstotzingen (1, 3A, 6, 9, 12B, and 12C, hereafter referred to as Niederstotzingen North) have the greatest affinity with modern
northern, eastern, and central Europeans, while individuals 3B and 3C (hereafter referred to as Niederstotzingen South) have the greatest affinity with southern Europeans and individuals from the eastern Mediterranean (Fig. 2). Admixture analysis with five components that had the optimum cross-validation (CV) error (table S12) indicates that Niederstotzingen North individuals have the most similar admixture components to modern-day eastern European populations (fig. S5). Niederstotzingen South individuals have a proportion of components that most resemble modern Mediterranean populations. However, 3B and 3C do not have similar proportions of ancestry to one another unlike Niederstotzingen North individuals.

To formally test the extent of shared genetic drift among individuals, outgroup F3 statistics were applied to the autosomal data using populations guided by PCA projections. F3 statistics (Mbuti; Niederstotzingen individual, modern west Eurasian) concur with PCA and admixture estimates, showing that Niederstotzingen North individuals are closely related to northern and eastern European populations, particularly from Lithuania and Iceland. Niederstotzingen South individuals have the greatest affinity to southern Europeans (figs. S6 to S13), in particular to populations from modern northern Spain, but have a much weaker population affinity to any European population overall, thus suggesting recent admixture between different populations in their ancestry.

**Estimation of kinship**

To investigate kinship between the individuals, we made pairwise estimates of first- and second-degree kinship based on the proportion of nonmatching autosomal genotypes (P0) in each unique pair from
all SNPs that overlapped with the 1240K (Fig. 3 and tables S15 and S16). READ (relationship estimation from ancient DNA) software defines first degree as immediate family (parent-offspring and siblings) and second degree as extended family (cousins, uncles/aunts, grandparent-grandchild, and half-siblings) and uses a normalized P0 value (27). READ software is tailored for aDNA from software designed for modern whole-genome sequencing data (28). To back up READ results, the coefficient of relatedness was estimated from non-normalized P0 without READ software (table S16) (16). Both kinship estimates show first-degree relatedness for pairs 1/3A, 1/6, 1/9, 3A/9, and 9/12B and second-degree relatedness for 1/12B, 3A/6, 3A/12B, and 6/9. Except for 12C, all of the Niederstotzingen North individuals are detectably and closely related. The Niederstotzingen South individuals are not detectably related to each other or any other members of the cohort.

**DISCUSSION**

The genetic characterization of the individuals at Niederstotzingen offers insights into their kinship structure and origin. We show genome-wide data for all 13 individuals recovered from the site, 8 of which (Fig. 1) had sufficient genomic enrichment to infer genetic affinity to a familial level. These results allow direct testing of historical and archeological hypotheses regarding the symbolism of burial rites from the Early Medieval.

Eleven individuals were probably genetically male. The combined 1240K and shotgun data inform the sex of the three infants (2, 4, and 5) and are consistent with the anthropologically determined sex of all adults, including three adults males with gracile craniofacial features that may be attributable to female osteology (3C, 6, and 12C) (8, 29). The observation that even most of the infants and juveniles were probably male and that grave 7 may have once contained females, which were later exhumed and possibly reburied (supported by the presence of women’s jewelry) (6), suggests that burial rites were sex-biased. Across the Merovingian Kingdom, male-only burials have been observed in this Adelsgräber buried in prominent places such as Roman roads (4). This pattern may reflect the military function of the burial or the social structure of the nobility. Likewise, sex-biased burial patterns have been observed at European sites throughout Late Antiquity (11, 30).

Genomically, the individuals buried at Niederstotzingen can be split into two groups: Niederstotzingen North (1, 3A, 6, 9, 12B, and 12C), who have genomic signals that most resemble modern northern and eastern European populations, and Niederstotzingen South (3B and 3C), who most resemble modern-day Mediterraneans, albeit with recent common ancestry to other Europeans. Niederstotzingen North is composed of those buried with identifiable artifacts: Lombards (individual 6), Franks (individual 9), and Byzantines (individuals 3A and 12B), all of whom have strontium and oxygen isotope signals that support local provenance (fig. S2) (8). Just two individuals, 3B (Niederstotzingen South) and 10 (no sufficient autosomal data, with R1 Y-haplogroup), have nonlocal strontium isotope signals. The $\delta^{18}O$ values suggest that individuals 10 and 3B may have originated from a higher-altitude region, possibly the Swiss-German Alpine foothills (8). Combined with the genome affinity of individual 3B to southern Europeans, these data provide

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**Fig. 3. Reconstruction of first- and second-degree relatedness among all related individuals.** Bold black lines and blue lines indicate first- and second-degree relatedness, respectively. Dark blue squares are identified males with age-at-death estimates years old (y.o.), mtDNA haplotypes, and NRY haplogroups. Red circles represent unidentified females that passed maternal haplotypes to their offspring. The light square represents one male infant that shares its maternal haplotype with individuals 12B and 9. N.D., not determined.
direct evidence for incoming mobility at the site and for contact that went beyond exchange of grave goods (4). Familia had holdings across the Merovingian Kingdom and traveled long distances to maintain them; these holdings could have extended from northern Italy to the North Sea. Nobles displayed and accrued power by recruiting outside individuals into the household as part of their traveling retinue. Extravagant burial rites of these familia are symbolic evidence of the Frankish power systems based on people Personenverbandstaaten imposed from the 5th until the 8th century CE (4). The assignment of grave goods and the burial pattern do not follow any apparent pattern with respect to genetic origin or provenance, suggesting that relatedness and fellowship were held in equal regard at this burial.

Further insights into the kinship structure are obtained from the reconstruction of direct relatives at the site. We demonstrated that five of the individuals (1, 3A, 6, 9, and 12B) were kin to at least second degree (Fig. 3 and tables S15 and S16); four of these were buried with distinguishable grave goods (discussed above and in fig. S1). These data show that at Niederstotzingen, at least in death, diverse cultural affiliations could be appropriated even within the same family across just two generations. This finding is somewhat similar to the burial of the Frankish King Childeric in the 5th century CE with a combination of Frankish and Byzantine grave goods that symbolized burial of the Frankish King Childeric in the 5th century CE with a distinct identity (Fig. 3 and tables S15 and S16); four of these were buried with distinguishable grave goods (discussed above and in fig. S1). These data show that at Niederstotzingen, at least in death, diverse cultural affiliations could be appropriated even within the same family across just two generations. This finding is somewhat similar to the burial of the Frankish King Childeric in the 5th century CE with a combination of Frankish and Byzantine grave goods that symbolized burial of the Frankish King Childeric in the 5th century CE with a distinct identity.

The burial of three unrelated individuals (3B, 3C, and 12C) in multiple graves beside the rest of the cohort would imply that this Alemannic group buried their dead based on a combination of familial ties and fellowship. One explanation could be that they were adopted as children from another region to be trained as warriors, which was a common practice at the time; these children were raised with equal regard in the familia (2, 4).

To infer a tentative family tree, we combined autosomal SNPs, NRY haplotypes, mtDNA haplotypes, and age-at-death data. It is possible that pairs 9/1, 9/3A, and 1/6 represent father-son relationships. The results also support that pairs 1/3A and 9/12B are siblings, as they share the same maternal haplotype (K1a and X2B4, respectively). Pairs 3A/6, 1/12B, 6/9, and 3A/12B are second degree–related, that is, they resemble uncles/cousins to sibling pair 1/3A. An infant buried in grave 4 has the same mtDNA haplotype (X2B4) as 9 and 12B, which supports a maternal relation, but there is no further evidence. The minimum mapping and base quality were both 30. MapDamage2.0 quantified deamination at 5’ and 3’ ends in non–UDG-treated libraries to show the presence of adNA (41). To authenticate the retrieved data, mtDNA and X chromosome contamination estimates were made with Schmutzi (42) and ANGSD (43), respectively. To investigate the impact that modern contamination may have had on the results, non–UDG-treated data were filtered with PMDtools (threshold 3) and analyzed in parallel with non–PMD-filtered reads (22).

To retrieve mtDNA haplotypes, stringent filtering with log2fasta (42) was applied (quality > 20) to create mitogenome sequences. mtDNA haplotypes were assigned with haplofind (phyloTree 17) (44, 45). The NRY haplotypes were identified relative to ISOGG database 11.349 (https://isogg.org/tree), and haplotyping was performed with ANGSD haploid caller (43) guided by yhaplo tool (46). Low-coverage (<2X) Y SNPs that were potentially caused by deamination were filtered. To estimate the sex of the individuals, the relative coverage of X and Y chromosomes was calculated from shotgun (23, 47) and 1240K capture (24) sequences.

To investigate genomic data, SAMtools mpileup (38) and PileupCaller (https://github.com/itschiff/SequenceTools/tree/master/src-pileupCaller) called pseudodiploid genotypes for the individuals, at loci that overlapped with the 1240K targeted SNPs, and merged them to a Human Origins Affymetrix (25) modern west Eurasian subset (n = 1063) (26). PCA smartpca (48, 49), admixture (50), and outgroup F3 statistics made estimates of ancestry from genome-wide data (25, 26). Admixture was run from 2 to 12 components (K), cross-validation (cv) with five replications each, and random seeding (-1 1000). The selection of the admixture results to present was based on the lowest CV error, the replicate with the highest log likelihood, and careful interpretation of the results (51). Kinship estimates used the READ tool and the coefficient of relatedness, which were adapted for low-coverage genotypes (16, 27).
SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/suppl/ADV_2018 Chỉ...
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