Genetic Kinship and Sex Determination of Early Modern Period Human Remains from a Defunct Graveyard in the Former Village of Obora (Located on Šporkova Street in Prague’s Lesser Town District)

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Abstract
The main aim of this study was to determine genetic kinship and genetic sex of individuals buried either in the same grave, multi-level grave, or neighbourhood grave. Success of genetic analyses is based on the quantity and quality of extracted aDNA, which can be compromised by degradation of DNA and possible contamination by modern DNA. We analysed archaeological skeletal remains from an Early Modern period graveyard belonging to the Church of St. John the Baptist in the former village of Obora, one of the most honourable Early Modern period archaeological sites in the Czech Republic. Most of the 906 excavated anatomically-laid burials are dated to the years 1730s–1770s. The results of 23 analysed individuals (divided into 4 groups) revealed that individuals are not blood relatives. Studies of historical written sources provide information that the parish affiliation at the time of death had a crucial role in choosing the place for burial. Genetic analyses increased success rate of sex determination to 91% compared to 61% determined by morphological methods. We were thus able to determine the genetic sex of children, an evaluation that cannot be made by morphological methods.

1. Introduction
The implementation of genetic analyses into studies of archaeological skeletal remains can provide information about genetic kinship (Ciu et al., 2015; Dégüilloux et al., 2014) and the genetic sex of children, when incomplete and poorly-preserved skeletons (Álvarez-Sandova et al., 2014; Lassen et al., 2000; Tierney, Bird, 2014) cannot be reliably determined with different methods. Analyses of ancient DNA (aDNA) have been previously used in demographic studies of skeletal archaeological remains from several archaeological sites in the Czech Republic, for example, by Boberová et al., 2012, Bravermanová et al., 2018, or Frolik et al., 2017. The determination of genetic kinship among the buried individuals would give an important insight into understanding funerary practices, and the social and demographic structures of historical cultures.

Additional useful information can also be obtained from written historical sources, such as civil and parish registers, testaments and chronicles.

The quality of genetic analyses of aDNA are negatively influenced by two major problems: its degradation into small fragments; and the contamination of aDNA with modern DNA. Firstly, over time, the DNA will become damaged and broken into small fragments due to its inhospitable environmental conditions (Hofreiter et al., 2001, pp. 353–354; Pääbo et al., 2004, pp. 654–660). Secondly, contaminant DNA can come from individuals who were in contact with the skeletal remains (archaeologists, anthropologists, or geneticists in the laboratory), as well as from chemical reagents, laboratory, or cross-sample contaminations. While working with our samples for genetic analyses, we followed the instructions published by Yang and Watt (2005).

Archaeogenetic research of genetic kinship is based on analyses of uniparental markers (Y-chromosome and mitochondrial DNA) and autosomal STR markers.
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Figure 2. Flowcharts by Jiří Vachuda. The flowcharts are parts of unpublished documentation of research in Šporkova Street, schematically representing the position of graves on the burial site in the geometrically defined sectors. Each sector is designated by a different colour. Some skeletons intersected more than one sector, and so are labelled using more than one colour. The results of the computer analysis of genetic kinship are the nearby flowcharts.

Figure 3. Skeletal remains of individuals H66 and H71 (in blue). Grave H66 contained skeletal remains of an adult female with additional bones (in green) of a child and adult male. Photographed by Jiří Vachuda.
Table 1. The list of individuals analysed.

| No. | ID     | Sex determined anthropologically | Age determined anthropologically | Analysed part of skeleton | Concentration of aDNA (pg/ul) | Inventory number | Group |
|-----|--------|----------------------------------|----------------------------------|---------------------------|-------------------------------|------------------|-------|
| 1.  | H72/I  | female                           | 30–40                            | tooth                      | 33.4                          | P7A 19 001       | 1     |
| 2.  | H72/II | female                           |                                   | bone                       | 4.64                          |                  |       |
| 3.  | H78/I  | undetermined                     | 2–3                              | tooth                      | 1.55                          | P7A 19 007       | 1     |
| 4.  | H78/II | undetermined                     |                                   | bone                       | 36                            |                  |       |
| 5.  | H94/I  | undetermined                     | juvenis                          | tooth                      | 4.22                          | P7A 19 021       | 1     |
| 6.  | H94/II | undetermined                     |                                   | bone                       | 5.25                          |                  |       |
| 7.  | H96/I  | female                           | 50–60                            | metatarsal                 | 0.24                          | P7A 19 023       | 1     |
| 8.  | H96/II | female                           |                                   | rib                        | 8.03                          |                  |       |
| 9.  | H98/I  | female                           | 30–40                            | tooth                      | 2.46                          | P7A 19 025       | 1     |
| 10. | H196/I | male                             | maturus                          | tooth                      | 15.3                          | P7A 19 122       | 1     |
| 11. | H196/II| male                             |                                   | rib                        | 11.6                          |                  |       |
| 12. | H222/I | male                             | 40–50                            | ulna                       | 394                           | P7A 19 162       | 1     |
| 13. | H222/II| male                             |                                   | tooth                      | 366                           |                  |       |
| 14. | H251/I | female                           | 30–40                            | tooth                      | 2.11                          | P7A 19 173       | 1     |
| 15. | H251/II| female                           |                                   | tooth                      | 0.9                           |                  |       |
| 16. | H120/I | undetermined                     | newborn                          | ulna                       | 9.06                          | P7A 19 047       | 1     |
| 17. | H120/II| undetermined                     |                                   | rib                        | 66                            |                  |       |
| 18. | H242/I | undetermined                     | juvenis                          | bone                       | 4.32                          | P7A 19 162       | 1     |
| 19. | H242/II| undetermined                     |                                   | rib                        | 2.12                          |                  |       |
| 20. | H64/I  | undetermined                     | 18 months                        | tooth                      | 1.26                          | P7A 18 993       | 2     |
| 21. | H64/II | undetermined                     | 18 months                        | bone                       | 2.54                          |                  |       |
| 22. | H65/I  | undetermined                     | newborn                          | humerus                    | 35.7                          | P7A 18 994       | 2     |
| 23. | H65/II | undetermined                     |                                   | rib                        | 68.1                          |                  |       |
| 24. | H101/I | female                           | maturus                          | tooth                      | 47.4                          | P7A 19 028       | 2     |
| 25. | H101/II| female                           |                                   | rib                        | 1.25                          |                  |       |
| 26. | H66a/I | female                           | maturus                          | tooth                      | 6                             |                  |       |
| 27. | H66a/II| female                           |                                   | tooth                      | 17.8                          |                  |       |
| 28. | H66/b/I| undetermined                     | juvenis                          | rib                        | 11.4                          | P7A 18 995       | 3     |
| 29. | H66/b/II| undetermined                    |                                   | bone                       | 2.04                          |                  |       |
| 30. | H66/e/I| male                             | maturus                          | tooth                      | 2.79                          |                  |       |
| 31. | H66/e/II| male                            |                                   | tooth                      | 31.3                          |                  |       |
| 32. | H71/I  | undetermined                     | newborn                          | femur                      | 2.62                          | P7A 19 000       | 3     |
| 33. | H71/II | undetermined                     |                                   | os petrosum                | 69.2                          |                  |       |
| 34. | H85/I  | female                           | 20–30                            | tooth                      | 0.5                           | P7A 19 014       | 3     |
| 35. | H85/II | female                           |                                   | tooth                      | 5.95                          |                  |       |
| 36. | H85/III| female                           |                                   | tooth                      | 2.16                          |                  |       |
| 37. | H82/I  | undetermined                     | newborn                          | radium                     | 3.8                           | P7A 19 011       | 4     |
| 38. | H82/II | undetermined                     |                                   | phalang                    | 2.93                          |                  |       |
| 39. | H97/I  | female                           | 50–60                            | calf bone                  | 2.49                          | P7A 19 024       | 4     |
| 40. | H97/II | female                           |                                   | phalang                    | 1.29                          |                  |       |
| 41. | H165/I | female                           | 40–50                            | tooth                      | 35.2                          | P7A 19 092       | 4     |
| 42. | H165/II| female                           |                                   | os petrosum                | 396                           |                  |       |
| 43. | H145/I | male                             | adultus                          | os petrosum                | 317                           | P7A 17 072       | 4     |
| 44. | H145/II| male                             |                                   | tooth                      | 17.5                          |                  |       |
| 45. | H192/I | male                             | adultus                          | tooth                      | 24.9                          | P7A 19 118       | 4     |
| 46. | H192/II| male                             |                                   | metacarpus                 | 1.97                          |                  |       |
see Figure 3). Samples of teeth and bones were taken from different parts of the skeletons, depending on their state of skeletal preservation. For detailed information about the samplings, see Table 1. Sampling took place in the National Museum in Prague, where the remains are deposited.

Samples were analysed in several independent steps in four separate rooms (mechanical cleaning; extraction of aDNA; quantification and PCR amplification; post-PCR sequencing). Blank controls were added to each step/reaction to monitor for possible contamination resulting from the lab procedures, but revealed no evidence thereof.

2.1 Mechanical cleaning and extraction of aDNA

Samples were rinsed using 96% ethanol and ultra clean water. Bone and teeth surfaces were sanded using either a Dremel Multi (Dremel) electric mini sander, or manually using sandpaper due to a sample’s preservation. Bones were cut into small pieces and ground into powder using a 6870 Freezer Mill (Spex Sample Prep). Subsequently, 70mg of the bone powder was incubated in a lysis buffer (0.5M EDTA [pH 8.0], Proteinase K and 0.5% SDS) at 56°C in a UVP HB –1000 Hybridizer Hybridization Oven (Analytik Jena US LLC) for 24 hours. Finally, aDNA was extracted using a MinElute PCR Purification Kit (Qiagen) according to a modified protocol published by Yang et al. (1998) and Anderung et al. (2008).

2.2 Quantification of aDNA

The success of the extraction of preserved aDNA and the amount of extracted aDNA was determined using a real-time PCR quantification Plexor HY System (Promega) kit on a LightCycler 480 RealTime PCR Instrument (Roche). Samples were prepared in duplex reactions. The Plexor HY kit contains primer for a target of a 133bp sequence from a testis-specific protein, Y-encoded (TSPY) locus on chromosome Y, providing a means of determining genetic sex.

2.3 Amplification and sequencing of aDNA

Samples were analysed for 23 autosomal STR markers, amelogenin X and Y, and 23 Y-chromosomal STR markers using four commercially-available kits: the PowerPlex ESX 17 System, the PowerPlex ESI 17 Pro System, the PowerPlex 16 System, and the PowerPlex Y23 System (all from Promega Corporation). Amelogenin X and Y loci were used to determine genetic sex. Each sample was analysed in several independent amplifications and sequencing reactions using peqSTAR 96X Universal Gradient cycler (VWR Peclab) and the Applied Biosystems 3130xl Genetic Analyzers instrument (15kV injection for 15s at POP4 polymer) (Applied Biosystems). Samples were prepared according to the manufacturer’s recommendation with 32 amplification cycles instead of the 30 that were recommended by the manufacture protocol.

2.4 Data analyses

Raw data from capillary electrophoresis were analysed with GeneMapper IDX software (Applied Biosystems). Results of autosomal and Y-chromosomal STR markers were used for genetic kinship and genetic sex determination among buried individuals. Results of STR markers were evaluated and computed by several software programs: Mlrelate (Kalinowski et al., 2006), Familias 3 (Kling et al., 2014), Network 5 (Bandelt et al., 1999), and Network Publisher 2.1.2.5 (Fluxus Technology LtD.) A phylogenetic network of Y-chromosomal STR markers was constructed only for markers that were successfully genotyped in all male samples. The network was constructed using Median joining (Bandelt et al., 1999), and the final tree was redrawn by Network Publisher 2.1.2.5 (Fluxus Technology LtD.). Genetic kinship between samples in all groups was computed by one to one for all samples using software Familias 3, that differentiated between five categories of genetic relationship (parent-offspring, full siblings, half siblings, cousins and second cousins) as well as unrelated individuals using Blind search by calculating a likelihood ratio (Kling et al., 2014) and ML-Relate (Kalinowski et al., 2006) that determined three close relationships (parent-offspring, full siblings and half siblings) and unrelated individuals (Kalinowski et al., 2006).

3. Results

The success of the genetic analyses depends on the quantity and quality of the extracted aDNA (Table 1). Genetic sex and genetic kinship was evaluated for individuals that were successfully genotyped in several independent reactions of two or three different samples from one individual. Samples H64 and H94 were excluded from the statistical analyses, since sample H64 failed to be successfully genotyped, and sample H94 did not provide reliable results (results from two different parts of the skeleton gave different results). The skeletal remains of H94 were excavated during two different archaeological excavations and it is possible that the two parts of the skeleton were completed incorrectly.

3.1 Genetic kinship

Genetic kinship was determined from the result of autosomal (Table 2) and Y chromosomal STR markers (Table 3). STR profiles listed in Table 2 and Table 3 are summaries of all performed analyses from all kits, as well as all samples analysed for the same individual. The success rate of STR marker detection was increased using three different available autosomal kits. Ancient DNA is degraded into small fragments over time, and it is necessary to analyse small fragments (Allentoft et al., 2012; Pääbo, 1989). The advantage of using the PowerPlex ESX 17 System and PowerPlex ESI 17 Pro System kits as complements is that while they contain the same markers, the primers are designed to complement one another, with a different final marker length. Markers that are long in the first kit are short in the second. The stratigraphic relation between individuals within the burial grounds is shown on flowcharts below (Figure 2). Genetic analyses revealed only unrelated
### Table 2. Results of autosomal STR markers (NA – results not available; XX – female; XY – male).

| Sample | D22S1045 | D2S1338 | D18S51 | D1S1656 | TH01 | D10S1248 | vWa | D2S441 | D21S11 |
|--------|-----------|---------|--------|---------|-------|-----------|-----|---------|--------|
| H72    | 16; 17    | 8; 9    | 13; 16 | 17; 19  | 15; 17| 14; 15    | 30  | 17; 19  | 15; 17 |
| H74    | 16; 17    | 8; 9    | 13; 16 | 17; 19  | 15; 17| 14; 15    | 30  | 17; 19  | 15; 17 |
| H94    | 16; 17    | 8; 9    | 13; 16 | 17; 19  | 15; 17| 14; 15    | 30  | 17; 19  | 15; 17 |
| H98    | 16; 17    | 8; 9    | 13; 16 | 17; 19  | 15; 17| 14; 15    | 30  | 17; 19  | 15; 17 |
| H106   | 16; 17    | 8; 9    | 13; 16 | 17; 19  | 15; 17| 14; 15    | 30  | 17; 19  | 15; 17 |
| H222   | 16; 17    | 8; 9    | 13; 16 | 17; 19  | 15; 17| 14; 15    | 30  | 17; 19  | 15; 17 |
| H251   | 16; 17    | 8; 9    | 13; 16 | 17; 19  | 15; 17| 14; 15    | 30  | 17; 19  | 15; 17 |

### Table 3. Results of Y-chromosomal STR markers.

| Sample | DYS549 | DYS533 | DYS437 | DYS438 | DYS570 | DYS391 | DYS635 | YGATA–H4 | DYS19 |
|--------|--------|--------|--------|--------|--------|--------|--------|-----------|--------|
| H72    | NA     | NA     | NA     | NA     | NA     | NA     | NA     | NA        | NA     |
| H74    | NA     | NA     | NA     | NA     | NA     | NA     | NA     | NA        | NA     |
| H94    | NA     | NA     | NA     | NA     | NA     | NA     | NA     | NA        | NA     |
| H98    | NA     | NA     | NA     | NA     | NA     | NA     | NA     | NA        | NA     |
| H106   | NA     | NA     | NA     | NA     | NA     | NA     | NA     | NA        | NA     |
| H222   | NA     | NA     | NA     | NA     | NA     | NA     | NA     | NA        | NA     |
| H251   | NA     | NA     | NA     | NA     | NA     | NA     | NA     | NA        | NA     |
| H82    | NA     | NA     | NA     | NA     | NA     | NA     | NA     | NA        | NA     |
relationships between analysed samples within all groups. The matrix generated by MI-Relate software (Figure 2), provides information that individuals in all groups are not blood-related.

Six individuals were determined by a signal for amelogenin Y as a male and were analysed for Y-chromosomal STR markers (Table 3). All identified male individuals differed considerably from each other in terms of observed alleles (Figure 4), providing no evidence of any father-son relationships, nor of a common close male ancestor.

![Figure 4](image)

**Figure 4.** The phylogenetic network constructed for Y-chromosomal STR markers (yellow rings—individuals; red numbers—number of mutations).

### 3.2 Sex determination

The presence/absence of a signal for amelogenin Y locus was used to determine the genetic sex of skeletal remains (XX – female and XY – male). Six individuals determined as a male by the presence of a signal for amelogenin Y were successfully genotyped for Y-chromosomal STR markers. Due to the fact that the amelogenin Y locus can be affected by allelic drop-out, we also took the result of the amplification TSPY gene in the Plexor HY kit into consideration. All samples that did not have a signal for amelogenin Y were also not amplified for the Y-chromosomal TSPY gene, and genetic sex was classified as a female (Table 4). Our results were compared with the morphological findings of studies performed by Milan Stloukal from the Department of Archaeology of the National Museum in Prague. His unpublished morphological examinations of skeletal remains are archived in the Department of Archaeology in the National Heritage Institute in Prague. The anthropological sex and age of the skeletal remains were determined using methods in accordance to the protocol by Ferembach et al. (1979). We observed two cases of discordance (for individuals H145 and H165) between morphological and genetic findings (see Table 4), and thus were able to increase the rate of sex determination from 61% for morphological findings (14 individuals) to 91% (21 individuals) for genetic findings. We were also able to determine the sex of children that could not be evaluated by morphological methods. Both individuals H145 and H165 were poorly preserved, having seriously damaged skeletons and fragmented skulls.

**Table 4.** Results of morphological and genetic sex determination; discordances between morphological and genetic findings are labelled in red.

| No. | ID  | Sex determined anthropologically | Sex determined genetically | No. | ID  | Sex determined anthropologically | Sex determined genetically |
|-----|-----|----------------------------------|---------------------------|-----|-----|----------------------------------|---------------------------|
| 1.  | H72 | female                           | female                    | 13. | H101| female                           | female                    |
| 2.  | H78 | undetermined                     | female                    | 14. | H66a| female                           | female                    |
| 3.  | H94 | female                           | undetermined              | 15. | H66b| undetermined                     | female                    |
| 4.  | H96 | female                           | male                      | 16. | H66c| male                             | male                      |
| 5.  | H98 | female                           | female                    | 17. | H71 | undetermined                     | female                    |
| 6.  | H196| male                             | male                      | 18. | H85 | female                           | female                    |
| 7.  | H222| male                             | female                    | 19. | H82 | undetermined                     | male                      |
| 8.  | H251| female                           | female                    | 20. | H97 | female                           | female                    |
| 9.  | H120| undetermined                     | female                    | 21. | H165| male                             | female                    |
| 10. | H242| undetermined                     | female                    | 22. | H145| male                             | female                    |
| 11. | H64 | undetermined                     | undetermined              | 23. | H192| male                             | male                      |
| 12. | H65 | undetermined                     | female                    |     |     |                                   |                           |

**4. Discussion**

The results of the genetic analyses confirmed the hypothesis about the funerary practices of Early Modern period burghers, which was based on the study of historical written sources such as death registers, parish registers and testaments. Historical written sources did not provide any clear information about the existence of family graves on the bourgeois graveyard of St. John the Baptist church in the in Early Modern period village of Obora. Genetic analyses revealed that the individuals, who were buried in the same...
multi-level graves or in neighbouring graves, are not blood-related members of a single family.

The evidence from parish and civil registers suggests that bourgeois (middle class) members of society were buried in accordance to the parish affiliation of the given house which was the site of their death. Division of a family at the time of death was not unusual in that period. As a case in point, we can mention the burials of the young boy Johann Rolllaw and his mother. Although the boy died on 3rd May 1764 and his mother on 22nd May 1764, they were not buried in the same grave, nor even at the same graveyard as a result of the different parish affiliation of the houses in which their death took place. The boy died at the "House of Three Swallows" and was buried at the graveyard of the Church of St. John the Baptist, but his mother died at "Schumann House", and was buried at the graveyard of the Church of St. Vavrinec. Her daughter Rosina died in October 1764 at the house "U Jedličků" and was buried at the graveyard of the Church of the St. Vavrinec (Prague City Archives, Collection of Matrices, sign MK Z4, fold 19–20 and 22; Omelka, Řebounová, 2012a, p. 239). A very similar example was in the case of the Roßenfeld family. Three of the five children were buried at the graveyard of the Church of St. Vavrinec and two at the graveyard of the Church of St. John the Baptist. It was also probably due to their different parish affiliations (Prague City Archives, Collection of Matrices, sign MK Z3, fold 310 and 313; sign MK Z4, fold 20 and 31).

Other useful sources of funerary practices can be gleaned from testaments. Testaments of aristocratic and the richest, social-bourgeoisie class individuals, who were usually buried in church interiors, were written very precisely, containing detailed information about where exactly they want to be buried and even with whom they wish to be buried after death (Král 2005; Nováčková et al., 2019, in press). The graveyard of the Church of St. John the Baptist was mainly used to bury citizens belonging to the middle or lower-middle social classes, and individual testaments (if written) usually only specified the name of the church. We can mention, for example, the testament of František Dispačch. He died in 1766 in his house in Lesser Town, which belongs to the parish district of the Church of St. John the Baptist. His testament was written only several months before his death, and he wanted to be buried at the graveyard of the Church of St. John the Baptist or the graveyard of the Church of St. Wenceslas. Finally, he was buried at the graveyard of the Church of St. Wenceslas (Manuscript Collection, sign 4764, fol. A21–A22) and not at the graveyard of the Church of St. John the Baptist, where his five children had been buried before him (Anna died in 1731, Vaclav in 1737, Theresie in 1743, Ludmila in 1743, and Antonie in 1750). All his children died in their father’s house (Prague City Archives, Collection of Matrices, sign MK 3, fol. 185, 215, 253, 256 and 281). There is also mention of Anna Dispačhova, who died in 1742 in the house “At the White Angel”, and was buried at the graveyard of the Church of St John the Baptist, but the relationship with František Dispač is not clear from the register (Archive of the City of Prague, Collection of Matrices, sign MIK 3, fol. 236). It is evident that family relationships were not taken into consideration when members of one family were buried.

According to the data from historical written sources and from the results of genetic analyses, there is no indication that people from the Early Modern period’s lower and middle social classes (i.e. most of the people buried at graveyard of the Church of the St. John the Baptist) of purposefully-buried members of one family were buried in one multi-level grave, or in neighbourhood grave sites, or even in the same graveyard, as was the very common practice of the aristocracy and the richest among the population (Král, 2005).

5. Conclusion

In this study we have applied an interdisciplinary approach to investigate kinship, genetic sex and the funerary practices of an Early Modern period bourgeois society. Genetic analyses are a powerful method for sex determination in skeletons of children, as well as in badly-preserved and incomplete skeletons of adults (Álvarez-Sandova et al., 2014; Lassen et al., 2000; Tierney, Bird, 2014), where morphological methods provide unreliable or no results (Álvarez-Sandova et al., 2014; Lassen et al., 2000; Tierney, Bird, 2014). We observed a contradiction between morphological and genetic methods in the sex determination of two separate skeletons of buried individuals: individuals H145 and H165 were poorly preserved, resulting in an unreliable morphological determination of sex. In such cases, genetic analyses are a more exact method to determine the sex of skeletal remains than are morphological treatments. By implementing genetic analyses, the number of successfully-determined individuals increased from 14 (61%) to 21 (91%); in addition, we were able to determine the genetic sex of children that could not be determined through morphological methods.

Genetic analyses are a crucial tool in determining the kinship of archaeological skeletal remains. The skeletal samples used for genetic analyses were chosen according to their relative stratigraphic positions within the burial grounds, and divided into four groups. Skeletons of adults and children buried in the same multi-level grave, or in very close proximity, have a high probability of being members of the same family. Genetic analyses of autosomal and Y-chromosomal STR markers revealed, however, that the individuals analysed were not blood-relatives. These results of genetic analyses are in accordance with and confirm the hypothesis based on the evidence provided by written historical sources (civil and parish registers and testaments). There is evidence that some members of families of middle and lower social classes were buried in different graveyards: because they had died in different houses belonging to a different parish affiliation. The tradition of founding family graves at that time is well documented among aristocratic families and the more wealthy inhabitants, who were usually buried together; however, this would be in the interior of the
church. On the other hand, the majority of baroque inhabitants of the past village of Obora buried at the graveyard of the Church of St. John the Baptist were probably buried there wherever a free place was available and according to their parish affiliation, without taking blood relationships into consideration.

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