

## Article

# Family History in the Iberian Peninsula during Chalcolithic and Bronze Age: An Interpretation through the Genetic Analysis of Plural Burials

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**Abstract:** Throughout history, it has been observed that human populations have buried the deceased members of their communities following different patterns. During the Copper Age and the Bronze Age—periods on which this study focuses—in the northern sub-plateau of the Iberian Peninsula, we identified different patterns of multiple or collective burial. This work analyzes a total of 58 individuals buried in different multiple or collective graves, to investigate whether the practice of these burials implies a family or biological link between individuals buried together. With this aim, STR markers of nuclear DNA were analyzed, as well as the hypervariable regions I and II of mitochondrial DNA, establishing both close kinship relationships and relationships through maternal lineage. We observed different burial patterns, detecting certain maternal lines preserved in some common burials maintained over time. Close family relationships were observed to a lesser extent, with some occasional exceptions. The results of the analysis formed the basis for a discussion on the concepts of family and community.

**Keywords:** ancient DNA; kinship analysis; family; Iberian Peninsula; bioarchaeology; paleogenetics



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## 1. Introduction

If we observe any modern cemetery, we can see that the burials are usually family-related; so, if individuals buried in the same grave were exhumed and analyzed genetically, we would surely find biological kinship. This could be close kinship, or at least biological lineage relations among the individuals buried together. There may be exceptions, such as couples buried together without descendants or other relatives; or the case of adopted children (Lozano-García et al. 2023). But in general, it is more usual to find family tombs with a biological link among the individuals buried together.

From this, we can deduce that, in today's culture, the weight of biological family ties in society, in general, is considerable. This would suggest that the organization of cemeteries may reflect the most important social relationships in our society. However, the image of the importance of the family in today's Western society may be poorly representative with regard to other cultures or periods, given that the concept of family may have varied, and this could be reflected in the burials (Gomes et al. 2021).

Likewise, if we transfer this idea to past times, the study of kinship relationships between individuals buried together in other past cultures, we could get an idea of the weight given to certain social relationships. We can learn whether the biological family was

also one of the key structures in society in other historical periods (Gomes et al. 2021), or whether, on the contrary, greater importance was given to other social relationships not related to biological ties.

In the case of a tomb in which we find different individuals buried together, there are different possible archaeological interpretations, which must be considered, including details such as stratigraphy, the superposition of bodies, bioarcheological studies, etc. First of all, we must discern whether it is a primary burial (when the skeleton is found in the original grave) or secondary burial (when the human remains have been transferred from a different original grave) (Aliaga 2012). Furthermore, regarding the number of individuals buried together, there are different possibilities: on one hand, there are collective burials, which consist of burial sites used over a long period (for example, megaliths or funerary caves), where human remains are deposited over time; these are similar to modern cemeteries, which are reopened every time a death occurs. On the other hand, there are cases of multiple individuals buried together at the same time, such as the graves used during a plague, or to bury the victims of a massacre or execution by firing squad.

In some particular cases, it can be difficult to determine whether the burial was simultaneous or not and these can be called plural burials. These can be, for example, double or triple burials, in which archaeologists must establish—during excavation and in the laboratory—whether the bodies were buried at the same time, or at intervals over a longer period.

Our research focuses on the human populations that lived during the 3rd and 2nd Millennia B.C. in the Duero basin (Central Spain), especially in its most central areas, where several individuals have provided sufficiently well-preserved, culturally characterized, and radiocarbon-dated samples to form the corpus of paleogenetic research. During this period in history, the different populations developed a variety of funeral rituals, many of them featuring collective burials.

The geographical area of interest for our study featured periods characterized by different materials (ceramics, metals, etc.) and funeral rituals, though with two main periods in terms of material culture: the Chalcolithic or Copper Age and the Middle/Late Bronze Age, subdivided into the phases Pre-Beaker and Beaker and Proto-Cogotas I and Cogotas I, respectively. As previously mentioned, different funeral traditions were implemented over time, alternating between collective, multiple, and individual burials. There were two historical moments of new population arrival: first, during the Pre-Bell Beaker period, associated with the K mitochondrial haplogroup; and second during Proto-Cogotas I culture, with other lineages of the H, HVO, and T haplogroups (Palomo-Díez et al. 2023).

During the studied periods, a trend is observed towards collective or multiple burials, in which different individuals are buried together. This work explores possible close kinship and maternal lineage between the individuals buried together in during the Third and Second Millennia B.C. in ten archaeological burial sites in the Duero basin (Central Spain).

## 2. Material and Methods

### 2.1. Material

A total of 58 individuals were analyzed from 10 different burial sites in the Duero basin (Central Spain), corresponding to 5 consecutive cultural periods. Table 1 summarizes the different archaeological sites by chronological period and the number of individuals from each grave analyzed.

**Table 1.** Relation of archaeological collective burials and individuals analyzed.

Chronological/Cultural Period	Archaeological Site	Localization	Kind of Burial	Number of Individuals
Pre-Bell Beaker	El Tomillar	Bercial de Zapardiel, Ávila, Spain	Multiple primary	8
	Los Areneros	La Lastrilla, Segovia, Spain	Multiple secondary	9
	Los Cercados	Mucientes, Valladolid, Spain	Multiple primary	3
Bell Beaker	Aldeagordillo	Aldeagordillo, Ávila, Spain	Collective	4
Middle Bronze Age	Cueva de la Revilla	Atapuerca, Burgos, Spain	Collective	8
Proto-Cogotas I	El Cerro de la Horra	La Horra, Burgos, Spain	Multiple	3
	Los Rompizales	Quintanadueñas, Burgos, Spain	Multiple primary	5
	Los Tolmos	Caracena, Burgos, Spain	Multiple primary	3
	Tordillos	Aldeaseca de la Frontera, Salamanca, Spain	Collective secondary	12
Cogotas I	La Requejada	San Román de Hornija, Valladolid, Spain	Multiple primary	3
Total number of individuals				58

The burial sites studied have different characteristics, as outlined below:  
Pre-Bell Beaker period:

- El Tomillar: two different plural burials were found. The first, burial 1, was a collective secondary burial. The second, burial 13, was a multiple primary burial. In this case, we analyzed the eight individuals buried in the multiple primary burial site ([Robledo and Trancho 2001](#); [Fabián-García 2001](#)).
- Los Areneros: a multiple secondary burial was analyzed, revealing nine individuals who had first been buried or laid exposed elsewhere. This was identified as a secondary burial site due to the fact that the individuals found were missing a series of bones. The final burial was performed all together, and it is impossible to know whether they all came from the same primary grave or site ([Delibes de Castro et al. 2007](#)).
- Los Cercados: the three individuals buried in the same multiple simultaneous primary burial were analyzed ([García Barrios 2007](#); [Palomo-Díez et al. 2017](#)).

Bell Beaker period:

- Aldeagordillo: a collective burial was found, composed of several successive burials in a cist ([Fabián-García 1991](#)).

Middle Bronze age:

- Cueva de la Revilla: like at Aldeagordillo, a collective burial site used over a long period was found in La Revilla Cave ([Abarquero et al. 2005](#); [Abarquero Moras 2005](#)).

Proto-Cogotas I culture:

- El Cerro de la Horra: an apparently triple burial was later identified as a simultaneous double burial, with two primary burials (the two smaller graves), and a short time later, a primary individual burial in a hole above the previous grave ([Palomino Lázaro et al. 1999](#); [Palomo-Díez et al. 2019](#)).
- Los Rompizales: quadruple simultaneous burial and an individual burial ([Antequem 2009](#); [Velasco Vázquez and Arroyo 2016](#)).
- Los Tolmos: triple simultaneous burial composed of two adult individuals and a perinatal individual (fetus or newborn). It was probably a double inhumation, where one of the adults was a pregnant woman ([Esparza-Arroyo et al. 2017](#); [Palomo-Díez et al. 2018](#)).
- Tordillos: secondary burials of incomplete remains were found in several pits, which seem to come from only two related primary burial sites ([Tejeda et al. 2012](#); [Esparza Arroyo et al. 2012](#)). Some remains may already have been buried in Cogotas I.

Cogotas I culture:

- La Requejada: triple (multiple) simultaneous burial ([Delibes de Castro 1978](#); [Esparza-Arroyo et al. 2012](#); [Palomo Díez et al. 2011](#)).

## 2.2. Methods

For the purpose of replicating the experimental procedure, we selected two teeth without cracks or cavities from each skeleton, according to the authenticity criteria of ancient DNA (aDNA) ([Pääbo et al. 2004](#); [Palomo Díez 2015](#)). In the case of the absence of teeth, we selected complete bone samples with good macroscopic appearance. The samples were cleaned using a Sand Blaster (Dentalarm Base 1 Plus) to remove exogenous DNA from the outer surface ([Palomo-Díez et al. 2018](#); [Palomo-Díez et al. 2023](#)). The samples were then irradiated with UV light (30 min) and transferred to sterile grinding vials. The grinding was performed in a Freezer Mill (SPEX Model 6700) and the resulting bone or teeth powder was stored at  $-20^{\circ}\text{C}$  until the DNA extraction was performed. For the DNA extraction, 250 mg of powder was taken from each sample, and the DNA extraction was performed according to the modified Rohland and Hofreiter protocol ([Rohland et al. 2010](#)). In the case of the Los Cercados archaeological site, the DNA was extracted using a non-destructive DNA extraction method ([Gomes et al. 2015](#)).

Genetic analyses were performed in an exclusive ancient DNA laboratory. Result reproducibility was assessed for every genetic marker by setting up two independent DNA extractions from each individual (from two different samples per individual) and at least two amplifications from each DNA extract and PCR amplification kit. To increase the number of markers, we used three different kits for the amplification of aSTRs (autosomal STRs): the AmpFLSTRs MiniFiler<sup>TM</sup> PCR Amplification Kit (MiniFiler<sup>TM</sup>) and AmpFLSTRVR NGM Select<sup>TM</sup> PCR Amplification Kit (NGM), both from ThermoFisher Scientific (Foster City, CA, USA), and the PowerPlexVR ESX SYSTEM (ESX) from Promega. The PCRs were carried out according to the kit manufacturer's recommendations ([Mulero et al. 2008](#); [Sprecher et al. 2009](#); [Green et al. 2013](#)). The STR amplicons were separated on an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, Waltham, MA, USA) and analyzed using GeneMapperVR software, version 4.0.

At least four PCRs were performed for each individual, two from each of the DNA extracts from the samples of each individual. A consensus genotype was reconstructed for each individual considering as valid the alleles that were obtained at least twice in two independent DNA amplifications from two different DNA extracts from each of the two samples from each individual. We considered only peaks over 50 RFUs in height. Exogenous DNA contamination of the samples was monitored by checking against the genetic profiles constructed for all the people involved in sample manipulation (Supplementary Material S1).

The LR value for different possible kinships was calculated using Familias 3 software ([Kling et al. 2014](#)), using the “Blind Search” tool. Due to the lack of allele frequencies for the original population, we used a modern Spanish DNA population database from the same region ([García et al. 2012](#)) to investigate possible relationships between individuals. Further possible relationships among individuals were tested using the Blind Search Tool of the Familias 3 software ([Kling et al. 2014](#)).

For mitochondrial DNA (mtDNA) sequence analysis, the hypervariable region I (HVRI) (positions 16105–16399) was analyzed using the primers designed by [Fernández \(2005\)](#); and 345 bp of the hypervariable region II (HVRII) (positions 55–400) were amplified according to [Martínez-Labarga and Rickards \(1999\)](#).

At least 8 PCRs were performed for each individual:

- Fragment 1 of HVI on Sample 1 of individual X
- Fragment 2 of HVI on sample 1 of individual X
- Fragment 1 of HVI on Sample 2 of individual X
- Fragment 2 of HVI on sample 2 of individual X
- Fragment 1 of HVII on Sample 1 of individual X
- Fragment 2 of HVII on sample 1 of individual X

- Fragment 1 of HVII on Sample 2 of individual X
- Fragment 2 of HVII on sample 2 of individual X

The consensus mtDNA profile of each individual was reconstructed from these short overlapping fragments, taking into account only the results replicated at least twice.

The PCR products of all the valid amplifications were cloned to improve the robustness of the results (Supplementary Material S2). This technique confirmed the absence of contamination and molecular damage.

We also considered the analysis of Y-chromosome STR markers, but the poor molecular conservation of the Y-chromosome did not allow us to obtain reliable results.

### 3. Results

Partial STR profiles were obtained (a partial profile being one with at least four STR markers without considering amelogenin) in 19 of the 58 individuals analyzed. This indicates a poor preservation of nuclear DNA, providing analyzable results in only 33% of cases. In the case of mitochondrial DNA, the results were better, with a haplotype of hypervariable regions I and II of mtDNA obtained in 40 of the 58 individuals (69%). Table 2 summarizes the global DNA analysis results.

**Table 2.** Global DNA analysis results (where a partial STR profile is one with at least four markers). #: number.

Chronological/Cultural Period	Archaeological Site	# Inhumed Individuals	# Partial STR Profiles Obtained	# mtDNA Haplotype Obtained
Pre-Bell Beaker	El Tomillar	8	0	0
	Los Areneros	9	4	7
	Los Cercados	3	3	3
Bell Beaker	Aldeagordillo	4	0	0
Middle Bronze Age	Cueva de la Revilla	8	1	8
Proto-Cogotas I	El Cerro de la Horra	3	0	3
	Los Rompizales	5	3	5
	Los Tolmos	3	3	3
Cogotas I	Tordillos	12	3	9
	La Requejada	3	2	2
Total number of individuals		58	19	40

The results are organized according to the different chronological periods. Supplementary Material S2 includes the autosomal STRs analysis results and mtDNA haplotypes obtained for each analyzed individual.

The results obtained gave us no information about the Bell Beaker period.

Table 3 shows the results of the kinship analysis using the Familias 3 “Blind Search” tool for the individuals inhumed in each of the plural burials, organized according to different chronological periods. Table 4 summarizes the main global results on close kinship and maternal lineage kinship.

Table 4 summarizes the main results.

**Table 3.** Kinship analysis was performed using the Familias 3 “Blind Search” tool for the individuals inhumed in each of the plural burials, organized according to the different chronological periods. The most probable kinships detected are marked in green; where probable kinship is established by at least 10 STR markers with or without shared mitochondrial DNA or, alternatively, 2 STR markers and shared mitochondrial DNA. The molecular sex of each individual is indicated in the second and third columns, ♀ for females and ♂ for males; “-” indicates cases, where it was not possible to determine the sex and the gender symbol, is in brackets (♀) or (♂) where the sex was determined anthropologically because molecular analysis was impossible.

Chronological Period	Person 1	Person 2	Relationship	Number of Markers Employed	LR	mtDNA Shared
Pre-Bell Beaker period	1ARE ♂	8ARE ♀	Parent–child	1	142.986	No
	6ARE ♂	8ARE ♀	Parent–child	1	142.986	No
	1ARE ♂	8ARE ♀	Half-siblings	1	71.9928	No
	6ARE ♂	8ARE ♀	Half-siblings	1	71.9928	No
	1ARE ♂	8ARE ♀	Siblings	1	71.7428	No
	6ARE ♂	8ARE ♀	Siblings	1	71.7428	No
	1ARE ♂	8ARE ♀	Cousins	1	36.4964	No
	6ARE ♂	8ARE ♀	Cousins	1	36.4964	No
	1ARE ♂	6ARE ♂	Cousins	11	16.7206	No
	1ARE ♂	6ARE ♂	Half-siblings	11	13.5692	No
	1ARE ♂	8ARE ♀	2nd Cousins	1	9.87411	No
	6ARE ♂	8ARE ♀	2nd Cousins	1	9.87411	No
	1CER ♀	3CER (♀)	Siblings	1	4.66881	No
	1CER ♀	3CER (♀)	Parent–child	1	3.32149	No
	1CER ♀	3CER (♀)	Half-siblings	1	2.16074	No
	1CER ♀	3CER (♀)	Cousins	1	1.58037	No
	1CER ♀	3CER (♀)	2nd Cousins	1	1.14509	No
Middle Bronze Age period	1REV ♂	2REV -	Half-siblings	3	1.65641	No
	1REV ♂	2REV -	Siblings	3	1.63107	No
	1REV ♂	2REV -	Cousins	3	1.51798	No
	1REV ♂	2REV -	2nd Cousins	3	1.15421	No
Proto-Cogotas I culture period	2RPZ ♀	5RPZ ♀	Half-siblings	2	2.22181	No
	2RPZ ♀	5RPZ ♀	Cousins	2	2.04135	No
	2RPZ ♀	4RPZ ♂	Cousins	7	1.80764	No
	2RPZ ♀	4RPZ ♂	Half-siblings	7	1.52991	No
	2RPZ ♀	5RPZ ♀	2nd Cousins	2	1.34105	No
	1RPZ ♂	4RPZ ♂	Cousins	6	1.04812	No
	LTB1 ♀	LTB3 ♀	Siblings	10	$1.47734 \times 10^6$	Yes
	LTB1 ♀	LTB3 ♀	Parent–child	10	$1.27541 \times 10^6$	Yes
	LTB1 ♀	LTB3 ♀	Half-siblings	10	43677.3	Yes
	LTB1 ♀	LTB3 ♀	Cousins	10	2399.52	Yes
	LTB1 ♀	LTB3 ♀	2nd Cousins	10	40.0288	Yes
	9TOR -	12TOR ♂	Parent–child	1	143.129	-
	9TOR -	12TOR ♂	Half-siblings	1	72.0643	-
	9TOR -	12TOR ♂	Siblings	1	71.8143	-
	9TOR -	12TOR ♂	Cousins	1	36.5321	-
	2TOR ♂	7TOR ♂	Siblings	2	34.8663	Yes
	1TOR ♂	2TOR ♂	Siblings	3	12.6649	No
	2TOR ♂	7TOR ♂	Parent–child	2	11.6005	Yes
	9TOR -	12TOR ♂	2nd Cousins	3	9.88303	-
	2TOR ♂	7TOR ♂	Half-siblings	2	6.67802	Yes
	1TOR ♂	10TOR -	Siblings	3	4.81948	No
	2TOR ♂	7TOR ♂	Cousins	2	3.93346	Yes
	3TOR ♂	10TOR -	Parent–child	1	3.89066	-
	6TOR ♂	7TOR ♂	Parent–child	2	3.66156	No
	1TOR ♂	2TOR ♂	Half-siblings	2	3.55878	No
	8TOR -	10TOR -	Siblings	1	3.3252	No
	1TOR ♂	7TOR ♂	Siblings	3	3.16624	No
	1TOR ♂	2TOR ♂	Cousins	2	3.04409	No
Cogotas I culture period	1LR (♀)	3LR ♀	Parent–child	2	14.0675	-
	1LR (♀)	3LR ♀	Siblings	2	11.4268	-
	1LR (♀)	3LR ♀	Half siblings		6.18456	-



**Table 4.** Summary of main results.

Chronological/Cultural Period	Archaeological Site (Individuals Number)	Kind of Burial	Close Kinship	Maternal Kinship
Pre-Bell Beaker	El Tomillar (0)	Multiple primary	No results	No results
	Los Areneros (7)	Multiple secondary	No	Yes
	Los Cercados (3)	Multiple primary	No	No
Bell Beaker	Aldeagordillo (0)	Collective	No results	No results
Middle Bronze Age	Cueva de la Revilla (8)	Collective	No results	No
Proto-Cogotas I	El Cerro de la Horra (3)	Multiple primary	No results	Yes
	Los Rompizales (5)	Multiple primary	No	Yes
	Los Tolmos (3)	Multiple primary	Yes	Yes
	Tordillos (9)	Some multiple secondaries pits	Yes	Yes
Cogotas I	La Requejada (3)	Multiple primary	No results	No

#### 4. Discussion

Regarding the kinship analysis among individuals inhumed together in the Pre-Bell Beaker period, in the Los Areneros archaeological site, the strongest probable kinship was identified among individuals 2ARE, 8ARE, and 20ARE. However, these are excluded because they all have fewer than four markers. A possible link between 1ARE and 6ARE was detected (a possible 2nd cousin kinship), which cannot be ruled out, given that even the mitochondrial DNAs match. The obtained LR (16.72) is very low, but since this is a cousin relationship, the LR could be considered a possible kinship. This would be the only possible relationship detected in this burial. It should also be noted that although the haplotypes do not match, all the individuals have the same haplogroup: the mitochondrial macrohaplogroup K.

No other type of kinship relationship is observed. But it is striking that the mitochondrial DNAs, despite not sharing identical haplotypes, are very similar, belonging to the same macrohaplogroup K. In these cases, it is possible that these individuals are distant relatives because they formed a part of an endogamic population maintained over time, which reused the same burial sites over a long period. Furthermore, there are some pairs of individuals which share mtDNA haplotypes, particularly individuals 1ARE with 20ARE (haplotype frequency of 128/40995 according to the EMPOP database), and 2ARE with 5ARE (haplotype frequency 14/40995 according to the EMPOP database). These coincidences could be an indication of maternal lineage, especially in the case of 2ARE and 5ARE, because of the low haplotype populational frequency. We must take into account the fact that the Los Areneros site is a multiple secondary burial, and despite the movement of the human remains, it would appear they shared maternal lineage ties; this suggests that family ties did influence the burial methods.

However, in the other archaeological site from the Pre-Bell Beaker period, Los Cercados, no close or lineage kinship was identified. In this case, we must remember that it was a ritual grave ([García Barrios 2007](#); [Palomo-Díez et al. 2017](#)), which could explain why the three individuals buried there did not have any biological connection. In this case, only three skulls were found (with no post-cranial remains) accompanied by the remains of numerous animals and other grave goods. These three women were probably buried together in a ritual celebration—perhaps a ritual sacrifice—and not due to any biological connections. The results obtained show that the three women were not related by maternal lineage, and we cannot say anything about close autosomal relations, because of the poor DNA preservation.

Moving on to the Middle Bronze Age period, we analyzed collective and multiple burials from four different archaeological sites. The first is La Revilla Cave, where the results obtained fail to reveal anything on close kinship (autosomal STRs). However, in terms of maternal lineage, we can state that the individuals buried in La Revilla Cave do

not share the same maternal lineage, instead presenting distinct mtDNA haplotypes. This clearly shows that, in the Cueva de la Revilla, the reason for burying these individuals together did not concern biological ties.

In the case of El Cerro de la Horra, we were unable to obtain enough data to determine close kinship. However, the mtDNA haplotypes are identical in the three individuals, which indicates that they could have shared the same maternal lineage; this is reinforced by the fact that the shared haplotype shows low frequency in the current population (2/41920). In the same way, in the Los Rompizales archaeological site, the poor state of nuclear DNA preservation meant we were unable to obtain reliable information on close kinship. Nevertheless, there could be some type of undetected relationship between individuals RPZ3 or RPZ5, given that the poor autosomal profiles do not allow for close kinship results to be determined. However, individuals 1 and 3 could be related through their maternal lineage. No other family ties through maternal lineage were found among the rest of the buried individuals.

The Los Tolmos archaeological site provided us with the best-preserved DNA, which allowed us to obtain reliable results. In this case, a close kinship relationship of mother–daughter was identified between individuals LTB1 and LTB3, which was also confirmed with mtDNA (Palomo-Díez et al. 2018). This could have been a pregnant woman who died before giving birth. The other individual (LTB2) does not have a close biological relationship with LTB1 and LTB3. However, the three women could belong to the same maternal lineage since number 2 only differs from the numbers 1 and 3 in one position of the mtDNA, which could be a point mutation.

In contrast to what Villalba-Mouco stated in his article on the El Argar society in the south of the Iberian Peninsula (Villalba-Mouco et al. 2022), in the northern sub-plateau, there is no evidence of double burials involving unrelated individuals that could be heterosexual couples.

Finally, the last site, from the period of the Proto-Cogotas I Culture, Tordillos, failed to produce enough autosomal STR results to determine closely related relationships with solid LRs. However, a possible close relationship (siblings, half-siblings, or cousins) was detected between individuals TOR2 and TOR7, who also share mtDNA, which would reinforce the maternal side of the relationship. However, it is the only case, since the mtDNA tells us that the rest of the inhumated individuals here do not share any maternal lineage.

The most recently studied chronological period was the Cogotas I Culture, with the only case of the La Requejada archaeological site, where we found incomplete autosomal STR profiles, insufficient to determine any close relationships. Regarding the maternal lineage, mtDNA profiles were obtained only for individuals 2 and 3, who are not related through maternal lineage.

In conclusion, we can state that only two close kinships were observed in all the plural burials studied: the case of Tordillos, where we observe a possible case of brotherhood, supported also by mtDNA; and the mother–daughter relationship in Los Tolmos. However, this second case was most likely a pregnant woman buried with another unknown woman with whom she did not have any close kinship, or to whom she was maybe related through maternal lineage. This means it cannot be interpreted as a burial of close family members, in any case; rather, it involved different social dynamics (Esparza-Arroyo et al. 2017).

However, maternal kinship is observed in many, if not most, cases, with the exception of the ritual burial at Los Cercados (García Barrios 2007; Palomo-Díez et al. 2017) and the funerary cave La Revilla, used over a long period. Finally, a triple simultaneous burial was found at the La Requejada archaeological site. Observing the results obtained in this work, we can state that a considerable number of burial sites from these periods show signs of social and/or religious rituals and traditions, unrelated to whether the individuals buried together had family connections.

Broadly speaking, we observed no differences in the burial patterns throughout the different chronological periods considered. Notwithstanding, in the case of collective tombs, it can be reasonably expected for individuals buried together to present blood ties,



given that this type of interment is typical for more modern family tombs or pantheons. This theory is confirmed in the case of Tordillos, as has also been observed in other early Bronze Age populations in southeastern Europe (Žegarac et al. 2021), also according to the mtDNA analysis (Žegarac et al. 2021; Knipper et al. 2017). Unfortunately, in the other collective burial cases analyzed (El Tomillar and Aldeagordillo), the results were insufficient due to poor DNA preservation. Nevertheless, the collective burial in the cave of La Revilla revealed no shared maternal lineage among the individuals; it could be interesting to research the Y chromosome paternal lineage for this site, although, the poor DNA template makes it difficult to obtain Y chromosome genetic profiles.

In the case of multiple burials, there is a greater diversity given that they can be due to the death of several individuals at the same time in a variety of circumstances (massacre, war, epidemic, etc.). In such cases, individuals may have been buried together without following the typical pattern of that society. This is observed at Los Cercados, where we find a triple simultaneous burial involving three people who were not biologically related, which is clearly a burial unrelated to any family ties. In the same way, at Los Tolmos, despite the identification of a maternal relationship, the two adult individuals were unrelated, of whom one merely happened to be pregnant.

In general, we have observed that the multiple secondary burials in various graves at Tordillos dating to the Proto-Cogotas I Culture seem to have been carried out over a long period by a community in which some individuals were linked by family ties. In one contemporary site, the La Revilla Cave, the collective burial also does not seem to have been carried out by a single family. These facts provide evidence of the probable exogamy practiced by the small communities of the Middle Bronze Age.

Regarding multiple burials in which different individuals were buried simultaneously, we can discern between small tombs with two or three individuals (such as Los Tolmos or Los Cercados), where we do not find close relationships or shared lineage, and El Cerro de la Horra, where the three buried people share the same maternal lineage. In the case of the larger multiple burials, such as Areneros and Rompizales, we also observed some maternal lineage relationships between the individuals, but no close kinship. This could point to the existence of populations with a certain degree of consanguinity, which would explain and lead to the conclusion that in both the Chalcolithic and the Bronze Age, beyond close kinship, belonging to a lineage was a very important factor.

In any case, each case needs to be analyzed individually, taking into account the archaeological, anthropological, and genetic characteristics in a holistic manner.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/genealogy8010025/s1>, S1: Exogenous DNA contamination of the samples was monitored by checking against the genetic profiles constructed for all the people involved in sample manipulation; S2: PCR products of all the valid amplifications.

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## References

- Abarquero, Francisco Javier, A. L. Palomino, and M. J. Negredo. 2005. La Cueva de la Revilla, un enterramiento colectivo del Bronce Protocogotas en la sierra de Atapuerca (Burgos). *Boletín del Seminario de Estudios de Arte y Arqueología*. Arqueología LXXI: 89–136.
- Abarquero Moras, Francisco Javier. 2005. *Cogotas I. La difusión de un tipo cerámico durante la Edad del Bronce (Monografías 4)*. Valladolid: Junta de Castilla y León. ISBN 84-9718-293-6.
- Aliaga, Almela Raquel. 2012. Términos y conceptos para el estudio de las prácticas funerarias en Arqueología. *Revista Historia Autónoma*. September 1. Available online: <https://dialnet.unirioja.es/descarga/articulo/4048996.pdf> (accessed on 10 January 2024).
- Antequem, S. L. 2009. Informe final de la excavación arqueológica en el yacimiento ‘Los Rompizales’. Proyecto constructivo Circunvalación de Burgos BU-30. Tramo Villalbilla de Burgos-Quintanadueñas (Provincia de Burgos). Informe Técnico depositado en el Servicio Territorial de Cultura (Junta de Castilla y León) de Burgos. Private Report.
- Delibes de Castro, Germán. 1978. Una inhumación triple de facies Cogotas I en San Román de Hornija (Valladolid). *Trabajos de Prehistoria* 35: 225–50.
- Delibes de Castro, Germán, Ángel Esparza Arroyo, Ángel L. Palomino Lázaro, and Javier Velasco Vázquez. 2007. Excavación arqueológica en Los Areneros de las Zumaqueras. La Lastrilla (Segovia). Informe técnico. Junta de Castilla y León. Private Technical Report. Available online: <https://dialnet.unirioja.es/servlet/articulo?codigo=3750838> (accessed on 10 January 2024).
- Esparza-Arroyo, Ángel, Javier Velasco-Vázquez, and Germán Delibes de Castro. 2012. Exposición de cadáveres en el yacimiento de Tordillos (Aldeaseca de la Frontera, Salamanca). Perspectiva bioarqueológica y posibles implicaciones para el estudio del ritual funerario de Cogotas I. BIBLID (0514-7336 (2012) LXIX, enero-junio pp. 95–128). Available online: [https://www.researchgate.net/publication/236612168\\_EXPOSICION\\_DE\\_CADAVERES\\_EN\\_EL\\_YACIMIENTO\\_DE\\_TORDILLOS\\_ALDEASECA\\_DE\\_LA\\_FRONTERA\\_SALAMANCA\\_PERSPECTIVA\\_BIOARQUEOLOGICA\\_Y\\_POSIBLES\\_IMPLICACIONES\\_PARA\\_EL\\_ESTUDIO\\_DEL\\_RITUAL\\_FUNERARIO\\_DE\\_COGOTAS\\_I](https://www.researchgate.net/publication/236612168_EXPOSICION_DE_CADAVERES_EN_EL_YACIMIENTO_DE_TORDILLOS_ALDEASECA_DE_LA_FRONTERA_SALAMANCA_PERSPECTIVA_BIOARQUEOLOGICA_Y_POSIBLES_IMPLICACIONES_PARA_EL_ESTUDIO_DEL_RITUAL_FUNERARIO_DE_COGOTAS_I) *Exposure* (accessed on 10 January 2024).
- Esparza-Arroyo, Ángel, Sara Palomo-Díez, Javier Velasco-Vázquez, Germán Delibes de Castro, Eduardo Arroyo-Pardo, and Domingo Salazar-García. 2017. Familiar kinship? Palaeogenetic and isotopic evidence from a triple burial of the Cogotas I archaeological culture (Bronze Age, Iberian Peninsula). *Oxford Journal of Archaeology* 36: 223–42. [CrossRef]
- Esparza Arroyo, Ángel, Velasco Vázquez, and Germán Delibes de Castro. 2012. HUM 2005-00139: *Planteamiento y primeros resultados de un proyecto de investigación sobre la muerte en Cogotas I*. Una cultura de la Edad del Bronce en la Península Ibérica: Homenaje a M<sup>a</sup> Dolores Fernández-Posse. Valladolid: Universidad de Valladolid, pp. 259–320.
- Fabián-García, Juan Francisco. 1991. El enterramiento campaniforme del túmulo 1 de Aldeagordillo (Ávila). *BSAA* 58: 97–135.
- Fabián-García, Juan Francisco. 2001. *El aspecto funerario durante el Calcolítico y los inicios de la Edad del Bronce*. Salamanca: Universidad de Salamanca. ISBN 84-7481-800-1.
- Fernández, Eva. 2005. Polimorfismos de ADN Mitocondrial en Poblaciones Antiguas de la Cuenca Mediterránea. Ph.D. thesis, Universidad de Barcelona, Barcelona, Spain.
- García Barrios, Ángel. 2007. *Un enfoque de género en la Arqueología de la Prehistoria Reciente del Valle Medio del Duero: Los cráneos femeninos calcolíticos de Los Cercados (Mucientes, Valladolid)*. en C. de la Rosa Cubo et al. (coords.): Nuevos enfoques para la enseñanza de la Historia: Mujer y Género ante el Espacio Europeo de Educación superior. Madrid: Asociación Cultural Almudayna, pp. 41–56.
- García, Oscar, J. Alonso, J. A. Cano, R. García, G. M. Luque, Pablo Martín, I. Martínez de Yuso, S. Maulini, David Parra, and Iñaki Yurrebaso. 2012. Population genetic data and concordance study for the kits Identifiler, NGM, PowerPlex ESX 17 System and Investigator ESSplex in Spain. *Forensic Science International: Genetics* 6: e78–e79. [CrossRef]
- Gomes, Cláudia, Sara Palomo-Díez, Ana María López-Parra, and Eduardo Arroyo-Pardo. 2021. Genealogy: The Tree Where History Meets Genetics. *Genealogy* 5: 98. [CrossRef]
- Gomes, Cláudia, Sara Palomo-Díez, Joan Roig, Ana María López-Parra, Carlos Baeza-Richer, Angel Esparza-Arroyo, Juan Gibaja, and Eduardo Arroyo-Pardo. 2015. Nondestructive extraction DNA methods from bones or teeth, true or false? *Forensic Science International: Genetics Supplement Series* 5: e279–e282. [CrossRef]
- Green, Robert L., Robert E. Lagacé, Nicola J. Oldroyd, Lori K. Hennessy, and Julio J. Mulero. 2013. Developmental validation of the AmpFSTR NGM SelectTM PCR Amplification Kit: A next-generation STR multiplex with the SE33 locus. *Forensic Science International: Genetics* 7: 41–51. [CrossRef]
- Kling, Daniel, Andreas Tillmar, and Thore Egeland. 2014. Familias 3—Extensions and new functionality. *Forensic Science International: Genetics* 13: 121–27. [CrossRef]
- Knipper, Corina, Alissa Mittnik, Ken Massy, Catharina Kocumaka, Isil Kucukkalipci, Michael Maus, Fabian Wittenborn, Stephanie Metz, Anja Staskiewicz, Johannes Krause, and et al. 2017. Female exogamy and gene pool diversification at the transition from the Final Neolithic to the Early Bronze Age in central Europe. *Proc. Natl. Acad. Sci. USA* 114: 10083–88. [CrossRef]
- Lozano-García, Manuel, Cláudia Gomes, Sara Palomo-Díez, Ana María López-Parra, and Eduardo Arroyo-Pardo. 2023. The Study of Adoption in Archaeological Human Remains. *Genealogy* 7: 38. [CrossRef]
- Martínez-Labarga, Cristina, and Olga Rickards. 1999. La utilización del DNA antiguo en la investigación de la historia evolutiva humana. *Revista Española de Antropología Biológica* 20: 195–213.
- Mulero, Julio, Chien Wei Chang, Robert Lagace, Dennis Wang, Jennifer Bas, Timothy McMahon, and Lori Hennessy. 2008. Development and validation of the AmpFISTR MiniFiler PCR Amplification Kit: A MiniSTR multiplex for the analysis of degraded and/or PCR inhibited DNA. *Journal of Forensic Sciences* 53: 838–52. [CrossRef]

- Palomino Lázaro, Ángel Luis, María José Negredo García, and Francisco Javier Abarquero Moras. 1999. Cabañas, Silos y Tumbas en el Yacimiento de El Cerro, La Horra (Burgos): A Vueltas sobre el Significado de un Campo de Hoyos en la Edad del Bronce de la Meseta. *Numantia* 7: 21–41.
- Palomo Díez, Sara. 2015. Caracterización genética de las poblaciones de las Edades del Cobre y del Bronce en la Submeseta norte de la Península Ibérica. Ph.D. thesis, Universidad Complutense de Madrid, Madrid, Spain. Available online: <https://docta.ucm.es/entities/publication/32487a5e-7248-449a-b01d-12f3a68aa3ef> (accessed on 10 January 2024).
- Palomo-Díez, Sara, Ángel Esparza-Arroyo, Cláudia Gomes, Olga Rickards, Javier Velasco-Vázquez, Ana María López-Parra, Carlos Baeza Richer, Cristina Martínez-Labarga, and Eduardo Arroyo-Pardo. 2017. Genetic characterization and determination of the number of individuals by molecular analysis in a prehistoric finding. *Forensic Science International: Genetics Supplement Series* 6: e487–e489. [CrossRef]
- Palomo-Díez, Sara, Ángel Esparza Arroyo, Mirian Tirado-Vizcaíno, Javier Velasco Vázquez, Ana María López-Parra, Cláudia Gomes, Carlos Baeza-Richer, and Eduardo Arroyo-Pardo. 2018. Kinship analysis and allelic dropout: A forensic approach on an archaeological case. *Annals of Human Biology* 45: 365–68. [CrossRef]
- Palomo-Díez, Sara, Ángel Esparza-Arroyo, Olga Rickards, Cristina Martínez-Labarga, and Eduardo Arroyo-Pardo. 2023. How Mitochondrial DNA Can Write Pre-History: Kinship and Culture in Duero Basin (Spain) during Chalcolithic and Bronze Age. *Genealogy* 7: 51. [CrossRef]
- Palomo-Díez, Sara, Carlos Baeza-Richer, Ángel Esparza-Arroyo, Javier Velasco-Vázquez, Cláudia Gomes, Alejandra Sánchez-Polo, Ana María López-Parra, Antonio Blanco-González, and Eduardo Arroyo-Pardo. 2019. Kinship analysis on skeletal ancient remains: The case of “El Cerro de la Horra” (Burgos, Spain). *Forensic Science International: Genetics Supplement Series* 7: 279–81. [CrossRef]
- Palomo Díez, Sara, Carlos Baeza-Richer, Eva Fernández-Domínguez, Cristina Gamba, Mirian Vizcaíno, and Eduardo Arroyo-Pardo. 2011. Análisis molecular de relaciones familiares en los restos hallados en dos tumbas de la Cultura Cogota I (Bronce Final). La Requejada (San Román de Hornija, Valladolid) y Los Tolmos (Caracena, Soria). In *Biodiversidad humana y Evolución*. Madrid: Spanish Society of Physical Anthropology, pp. 129–35, ISBN (impreso): 978-84-695-6322-9; ISBN (PDF): 978-84-695-6323-6.
- Pääbo, Svante, Hendrik Poinar, David Serre, Viviane Jaenicke-Després, Juliane Hebler, Nadin Rohland, Melanie Kuch, Johannes Krause, Linda Vigilant, and Michael Hofreiter. 2004. Genetic analyses from ancient DNA. *Annual Review of Genetics* 38: 645–79. [CrossRef]
- Robledo, Beatriz, and Gonzalo Tranco. 2001. Estudio bioantropológico de una sepultura múltiple. La tumba 13 de El Tomillar (Bercial de Zapardiel, Ávila). Dpto. Biología Animal (Antropología). Facultad de Biología. Universidad Complutense de Madrid. Available online: <https://www.seaf.es/images/seaf/papers/vol23/final%20v%2023%2001%20001-025.pdf> (accessed on 10 January 2024).
- Rohland, Nadin, Heike Siedel, and Michael Hofreiter. 2010. A rapid column-based ancient DNA extraction method for increased sample throughput. *Molecular Ecology Resources* 10: 677–83. [CrossRef]
- Sprecher, Cynthia, Robert McLaren, Dawn Rabbach, Benjamin Krenke, Martin Ensenberger, Patricia Fulmer, Lotte Downey, Erin McCombs, and Douglas R. Storts. 2009. PowerPlexVR ESX and ESI Systems: A suite of new STR systems designed to meet the changing needs of the DNA-typing community. *Forensic Science International: Genetics Supplement Series* 2: 2–4.
- Tejeda, Jesús Carlos Misiego, Miguel Ángel Martín Carbajo, Gregorio José Marcos Contreras, Francisco Javier Sanz García, and Francisco Javier Ollero Cuesta. 2012. Enterramientos humanos de la Edad del Bronce en el yacimiento de Tordillos (Aldeaseca de la Frontera, Salamanca): Un cementerio del horizonte Cogotas I. In *Una cultura de la Edad del Bronce en la Península Ibérica: Homenaje a M<sup>a</sup> Dolores Fernández-Posse*. Valladolid: Universidad de Valladolid, pp. 409–34.
- Velasco Vázquez, Javier, and Ángel Esparza Arroyo. 2016. Muertes ritualizadas en la Edad del Bronce de la Península Ibérica: Un enterramiento inusual en Los Rompizales (Quintanadueñas, Burgos). *Munibe Antropología-Arkeología* 67: 75–103. [CrossRef]
- Villalba-Mouco, Vanessa, Camila Oliart, Cristina Rihuete-Herrada, Adam B. Rohrlach, María Inés Fregeiro, Ainash Childebayeva, Harald Ringbauer, Iñigo Olalde, Eva Celdrán Beltrán, Catherine Puella-Mora, and et al. 2022. Kinship practices in the early state El Argar Society from Bronze Age Iberia. *Scientific Reports* 12: 22415. [CrossRef]
- Žegarac, Aleksandra, Laura Winkelbach, Jens Blöcher, Yoan Diekmann, Marija Krečković Gavrilović, Marko Porčić, Biljana Stojković, L. Milašinović, M. Schreiber, D. Wegmann, and et al. 2021. Ancient genomes provide insights into family structure and the heredity of social status in the early Bronze Age of southeastern Europe. *Nature Scientific Reports* 11: 10072. [CrossRef]

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