Megalithic tombs in western and northern Neolithic Europe were linked to a kindred society

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Paleogenomic and archaeological studies show that Neolithic lifeways spread from the Fertile Crescent into Europe around 9000 BCE, reaching northwestern Europe by 4000 BCE. Starting around 4500 BCE, a new phenomenon of constructing megalithic monuments, particularly for funerary practices, emerged along the Atlantic façade. While it has been suggested that the emergence of megaliths was associated with the territories of farming communities, the origin and social structure of the groups that erected them has remained largely unknown. We generated genome sequence data from human remains, corresponding to 24 individuals from five megalithic burial sites, encompassing the widespread tradition of megalithic construction in northern and western Europe, and analyzed our results in relation to the existing European paleogenomic data. The various individuals buried in megaliths show genetic affinities with local farming groups within their different chronological contexts. Individuals buried in megaliths display (past) admixture with local hunter-gatherers, similar to that seen in other Neolithic individuals in Europe. In relation to the tomb populations, we find significantly more males than females buried in the megaliths of the British Isles. The genetic data show close kin relationships among the individuals buried within the megaliths, and for the Irish megaliths, we found a kin relation between individuals buried in different megaliths. We also see paternal continuity through time, including the same Y-chromosome haplotypes reoccurring. These observations suggest that the investigated funerary monuments were associated with patrilineal kindred groups. Our genomic investigation provides insight into the people associated with this long-standing megalith funerary tradition, including their social dynamics.

paleogenomics | population genomics | migration | megalithic tombs

Investigations of the genetic relationships among humans from multiple Neolithic sites across western Eurasia have shown that Neolithic lifeways dispersed across Europe via a large-scale process of migration (1–6) starting from Anatolia and the areas of the Aegean at ca. 7000–6500 (cal) BCE (7–10). In Europe, migrating people and Neolithic lifeways dispersed along two main routes: an inland route (partly along the Danube River) and a route along Mediterranean coastal areas (11–13). Around 4000 BCE, Neolithic farming communities reached the northwestern fringes of Europe, including the British Isles (14, 15) and Scandinavia (1, 2, 16, 17). A marked hunter-gatherer (HG) admixture has been observed in the later farmer groups compared with the Early Neolithic farmers on the continent (2, 10, 12).

During this period of important social and demographic change, a new phenomenon of constructing megalithic monuments emerged, starting around 4500 BCE in France (18), 3700 BCE in the British Isles (14, 19–26), and 3600 in Scandinavia (16, 27). These Neolithic megalithic tombs are concentrated along the Atlantic coastal areas, stretching from the Mediterranean to Scandinavia, including the British Isles and regions in the northern European plain (28), but also in southern France, northern Italy, and on the Islands of Corsica and Sardinia (Fig. 1) (19, 27).

The emergence of these megaliths was closely associated with the development of farming communities (14, 23, 25, 27, 29), but the origin and the social structure of the groups are largely unknown. The similarities in the construction and design of some types of megaliths (i.e., dolmens and passage graves) from Iberia to southern Scandinavia, Britain, and Ireland is compelling. Interregional interaction has been evidenced in the same period from the dispersal of domesticated resources, raw materials, and artifacts, possibly reflecting shared social and cultural systems as well as shared cosmology of the groups (21, 27, 28, 30). Although it is clear

Significance

A new phenomenon of constructing distinctive funerary monuments, collectively known as megalithic tombs, emerged around 4500 BCE along the Atlantic façade. The megalithic phenomenon has attracted interest and speculation since medieval times. In particular, the origin, dispersal dynamics, and the role of these constructions within the societies that built them have been debated. We generate genome sequence data from 24 individuals buried in five megaliths and investigate the population history and social dynamics of the groups that buried their dead in megalithic monuments across northwestern Europe in the fourth millennium BCE. Our results show kin relations among the buried individuals and an overrepresentation of males, suggesting that at least some of these funerary monuments were used by patrilineal societies.
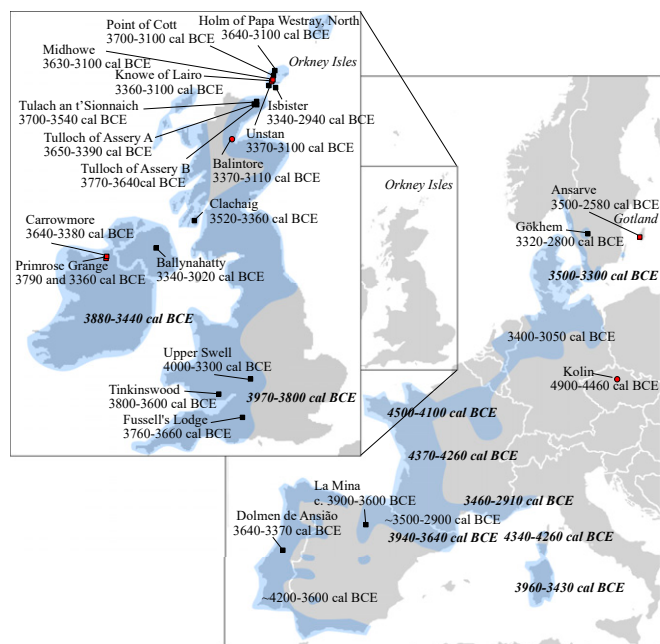
GENETICS

ANTHROPOLOGY

Fig. 1. Map of Europe with megalithic burial sites (red squares) and nonmegalithic sites (red circles) from this study, and comparative published data from megalithic sites (black squares) sequenced to date in Europe (Dataset S1.3). The date range represents the 95% CI of available samples from these sites, except for La Mina in Spain. Blue shading represents the estimated distribution of early megalithic burials. Bold italic type indicates dates (95% CI) estimated for the start of dolmens and passage grave monuments, based on samples from these contexts. Regular text indicates time interval associated with the earliest cultural material in the megaliths (27, 45).

that many megaliths were used for collective burials (27, 29, 31), it has been difficult to evaluate which members of the communities were buried in the tombs. Some assemblages include males, females, juveniles, and children, implying familial burials. Many tombs have poorly preserved human remains and also show secondary usage in later times, complicating assessments. The use of megaliths as burial grounds for the community as a whole would imply some level of shared ideology over vast geographical areas (31, 32). However, it has also been argued that the monumental burials and associated rich material culture reflect the emergence of social differentiation or stratification (33–36; see ref. 37 on segmentally structured societies), with the monuments perhaps symbolizing status and territorial markers (37–40).

Some scholars hypothesize that the people buried in the megalithic structures were kin related (41–43). Analyses of mitochondrial data (mtDNA) from megalithic burials at Falbygden and Gotland in modern-day Sweden have revealed a large lineage variation, and thus the groups did not seem to have been organized matrilineally (44, 45; however, contra ref. 43). Genomic data are necessary to provide deeper information on kin relations and the social dynamics and general social structure of the societies or groups. However, as genomic data have been available from only a few individuals from megalithic burials, the origin and dispersal dynamics of the funerary practices, as well as the population history of the people that used the burial constructions, have also remained uncertain.

In the present study, we investigated the genetic structure and demographic affinities of people buried within megaliths to shed light on this burial phenomenon, the social dynamics of the people buried in the monuments, and their demographic history. We generated and examined genome sequence data from 24 individuals from five megalith burial sites located in Ireland, the Orkney Isles, and the Island of Gotland in the Baltic Sea dated between ca. 3800 and 2600 cal BCE encompassing wide-ranging examples from the megalithic tradition in northern Europe. The study also incorporated three individuals from nonmegalith contexts

from mainland Scotland (3370–3100 cal BCE) and the Czech Republic (4825–4555 cal BCE) (Table 1).

Results

We present genome data from 27 individuals excavated from European Neolithic contexts, of whom 24 were buried in megaliths; Primrose Grange (n = 11) and Carrowmore (n = 1) in Ireland; Lairo (n = 1) and Midhowe (n = 2) in the Orkney Islands, Scotland; and Ansarve (n = 9) in the island of Gotland, Sweden (16, 45, 46) (Table 1 and *SI Appendix*, section S2). Individuals from the Scottish "short cist" burial Balintore (n = 1) and the Czech Republic Kolin Rondel site (n = 2) (46), associated with the Stroked Pottery culture, were also investigated. These individuals were all radiocarbon-dated to between 4825 and 2580 cal BCE (Table 1). We compared our data with genetic data previously generated from 36 individuals from 16 megalithic sites (Fig. 1 and Dataset S1.3), as well as with farmer groups of nonmegalithic contexts (Dataset S1.3), to investigate the population history of people buried in megaliths.

The individuals buried in these megaliths from the British Isles and Scandinavia show an ancestry similar to other contemporaneous farmer groups (Fig. 2*A*), with a majority of their ancestry related to early Neolithic farmers and a partial admixture component related to European Mesolithic HGs (Fig. 2*B*) (1, 2, 5–7, 10, 16, 46).

To further explore the demographic history of the individuals buried in the megaliths, we investigated the genetic affinities among sets of individuals and groups, using an f_3 outgroup test for groups of individuals buried in megalithic or nonmegalithic contexts, as well as between individuals from Atlantic coastal and inland Neolithic sites (*SI Appendix*, section S11.3 and Fig. S19). These analyses showed genetic associations between individuals from the same/similar geographic region and time period (Fig. 2*A* and *SI Appendix*, Figs. S16 and S17). However, some tests (*SI Appendix*, Fig. S19) indicated similar trends as shown in our principal component analysis (PCA) and previous studies (5, 11, 15, 47, 48) and suggested a demic connection among western European Neolithic groups to the exclusion of central European Neolithic groups, as well as a connection between the British Isles and Iberian groups (*SI Appendix*, section S11.4 and Figs. S20–S22). These results were not driven by greater levels of HG ancestry among the populations at the fringes of the Neolithic expansion (11, 12, 15, 16) (*SI Appendix*, section S11.4).

Interestingly, we also found a significant farmer-specific genetic affinity between the British Isles Neolithic populations and the Scandinavian populations (Ansarve and Gökhem; Fig. 1) to the exclusion of central European farmers (*SI Appendix*, Figs. S21 and S22). This observation is compatible with a further migration of farming groups along the European Atlantic coast, as has been suggested by the archaeological record (21, 49, 50).

We found that significantly more males than females were buried in the British Isles megaliths (31 of 42 randomly sampled individuals; P = 0.0014, binomial test) and at the Primrose megalith alone (9 of 11; P = 0.032) (*SI Appendix*, section S8). However, other megalithic tombs with at least four individuals investigated, including Ansarve (6 of 9; P = 0.25), Gökhem (1 of 4; P = 0.93), La Mina (2 of 4; P = 0.68), Holm of Papa Westray (2 of 4; P = 0.68), and Isbister (Tomb of the Eagles) (8 of 10; P = 0.054), did not show the same striking pattern, nor did nonmegalithic burials from the British Isles (15) (nonmegalithic burials: 6 of 10; P = 0.27, cave burials: 10 of 15; P = 0.27, both nonmegalithic and cave burials: 16 of 25; P = 0.11). Overall, genetic data from all individuals from megalithic contexts suggest a higher male-to-female ratio in these burial chambers (41 of 60; P = 0.0031) (*SI Appendix*, Table S3), although the tendency is similar (but not significant) for nonmegalithic burials (*SI Appendix*, section S8).

We found greater macrohaplogroup mtDNA diversity than Y-chromosomal (YDNA) diversity. Whereas mtDNA lineages from megalith burials harbor haplogroups K, H, HV, V, U5b, T, and J (among others), males from megalith burials belong almost exclusively to YDNA haplogroup I, more specifically to the I2a sublineage, which has a time to most recent common ancestor of ~15000 BCE (51). This pattern of uniparental marker diversity is

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Table 1. Summary of genetic and archaeological information about the 27 individuals in the study

Individual	Site	Radiocarbon date (95% CI, cal BCE)		Sequence coverage		Sex	Haplogroup		Estimated contamination		
		Upper	Lower	nuDNA	mtDNA		mt	Ychr	mtDNA	95% CI	Autosomal
Primrose 2	Primrose	3790	3660	5.76	817.93	XX	H1+16189		0.05	0.01–1.22	1.283
Primrose 17	Primrose	3780	3650	0.19	49.51	XY	K1a+195	I	0.66	0.11–21.63	0.049
Primrose 18	Primrose	3770	3650	0.10	55.71	XY	K1a+195	I	0.59	0.10–18.30	0.000
Primrose 12	Primrose	3770	3650	0.25	325.42	XY	W1+119	I2a2a1a1a2	0.09	0.01–2.62	0.000
Primrose 3	Primrose	3770	3650	0.22	125.69	XY	H1i	I	5.28	1.91–12.50	0.000
Primrose 16	Primrose	3690	3530	6.40	442.67	XY	K1a4a1	I2a2a1a1a	0.06	0.01–1.53	0.951
Primrose 10	Primrose	3640	3520	0.23	178.60	XY	K1a+195	I	0.17	0.03–5.23	0.000
Primrose 6	Primrose	3640	3380	0.27	1,158.06	XX	K1a+195		0.03	0.00–0.84	0.000
Primrose 13	Primrose	3630	3370	4.73	675.01	XY	T2b3c	I2a2a1a1a	0.03	0.01–0.64	1.731
Primrose 7	Primrose	3510	3360	0.01	43.44	XY	K1a4a1	NA	1.44	0.18–14.26	0.000
Primrose 9	Primrose	3500	3360	7.10	923.93	XY	U5b2c	I2a2a1a1a	0.03	0.00–0.88	1.520
Carrowmore 4	Carrowmore	3640	3380	0.04	451.69	XY	T2c1d1	I	0.03	0.00–0.72	0.100
Midhowe 1	Midhowe	3630	3370	0.27	22.00	XY	H5+16311	I2a1b	1.52	0.24–44.17	1.150
Lairo 1	Lairo	3360	3100	0.22	25.08	XY	U5b2	I2a1b	0.96	0.16–31.07	0.022
Balintore 4	Balintore	3370	3110	1.54	168.43	XX	H1		0.18	0.03–4.71	0.033
Midhowe 2	Midhowe	3360	3100	0.25	29.38	XY	K1a+195	I	0.75	0.14–23.16	0.281
Ansarve 5	Ansarve	3500[a]	3130*	0.13	114.73	XX	K1a2b*		0.21	0.04–7.79	0.000
Ansarve 3	Ansarve	3490[a]	3110*	0.14	300.87	XX	T2b8*		0.04	0.01–1.02	0.046
Ansarve 8	Ansarve	3340[a]	3030*	1.94	1,462.38	XY	J1c5*	I2a1b1a1[†]	0.01	0.00–0.14	0.441
Ansarve 14	Ansarve	3330[a]	2950*	2.58	431.47	XY	J1c5*	I2a1b1a1[†]	0.02	0.00–0.41	0.525
Ansarve 17	Ansarve	3330[a]	2930*	6.80	491.04	XY	HV0a*	I2a1b1a1[†]	0.06	0.01–2.06	1.461
Ansarve 6	Ansarve	3090[a]	2920*	0.0027	137.06	XY	J1c8a*	NA	0.06	0.01–1.70	NA
Ansarve 7	Ansarve	3010[a]	2890*	0.0014	24.54	XY	K2b1a*	NA	0.33	0.06–8.90	NA
Ansarve 9	Ansarve	2880[a]	2630*	0.0009	26.73	XX	K2b1a*		0.29	0.05–6.99	NA
Ansarve 16	Ansarve	2810[a]	2580*	0.33	23.17	XY	H7d*	I2a1b[†]	1.60	0.27–46.97	0.004
Kolin6	Kolin	4910	4740	1.51	218.40	XX	H+16129		0.10	0.02–2.23	2.639
Kolin2	Kolin	4650	4460	0.10	42.39	XX	W1+119		0.37	0.06–10.83	0.068

*Ref. 45.
[†]Ref. 16.

found not only among individuals buried in megaliths, but also in other farmer groups from the fourth millennium BCE, which display similar patterns of uniparental marker diversity (*SI Appendix,* Figs. S6 and S23) (10, 15, 48, 52). Some mtDNA lineages appear to be overrepresented at megalithic sites, with information from more than six individuals, including Primrose (n = 11; K1a+195 and K1a4a1 at 36% and 18% frequency, respectively), Ansarve (n = 9; J1c5 and K2b1a at ∼20% frequency), and Isbister (n = 10; K1a+195 at 20% frequency). Males from the present study belonged to YDNA haplogroup I, and those who could be resolved beyond this level were characterized as belonging to the I2a2a or I2a1b branch. Four of the 10 Primrose/Carrowmore males (Primrose 9, 12, 13, and 16) could be further resolved to the former sublineage, while the two Scottish males and the four Ansarve males could be further placed in the latter branch (Table 1 and *SI Appendix,* section S7).

Combining the YDNA lineages and the radiocarbon dates of the individuals, a possible scenario of paternal continuity is observed for the Primrose and Ansarve megaliths. From the Primrose site, Primrose 9, 13, and 16, separated in time by at least 1 generation and possibly up to 12 generations, display the I2a2a1a1a haplotype. In addition, the Primrose 3, 10, and 17 individuals were inferred to harbor variants common to the I2a2 lineage, although with low coverage support (*SI Appendix,* section S7). A similar scenario is observed for the Ansarve megalith, with the individuals Ansarve 8, 14, and 17, separated by at most a few generations, carrying haplotype I2a1b1a. Ansarve 16, dated to at least 100 y younger, shares variants along the I2a1b lineage (Table 1 and *SI Appendix,* section S7).

The high frequency of the HG-derived I2a male lineages among megalith as well as nonmegalith individuals (*SI Appendix,* section S11.6) suggests a male sex-biased admixture process between the farmer and the HG groups (2, 12, 53, 54), but when this admixture occurred is unclear. To characterize the extent of

sex-biased admixture between HGs and the individuals of the megalithic contexts, we assessed the affinity of all individuals buried in megaliths with sufficient genetic data, to an Early Neolithic farmer or a HG ancestry on the autosomes and the X chromosome using f4-statistics (*SI Appendix,* section S11.5). Higher levels of HG admixture on the autosomes than on the X chromosome implies a greater genetic contribution of male HGs than female HGs to these individuals, suggesting an HG male sex bias admixture. We find that in general, megalith groups do not harbor higher levels of HG ancestry on the autosomes compared with on the X chromosome (*SI Appendix,* Table S7 and Dataset S1.6), but the Scottish_MN farmers of this study showed a tendency toward an HG male-sex biased admixture in the recent past. The Scandinavian (Ansarve and Gökhem) individuals displayed an HG admixture for both the autosomes and the X chromosome (*SI Appendix,* Table S7), suggesting a scenario of more recent admixture with HGs in northern Europe.

Using READ (Relationship Estimation from Ancient DNA) software (55), we inferred six kin relationships among the megalith individuals of this study: five relations among the Irish megaliths (two first-degree and three second-degree connections) and a second-degree relation in the Ansarve tomb (Fig. 3 and *SI Appendix,* section S10). First-degree relationships are characterized by either parent-offspring or a full sibling relationship, second-degree kin connections are represented by half-siblings, grandparent-grandchild, aunt/uncle-niece/nephew, and double cousins. Combining the READ predictions, uniparental lineages, radiocarbon dating, and age at death if available for those individuals who could be assessed, we inferred the potential familial relationships (Fig. 3 and *SI Appendix,* sections S2, S6, S7, and S10). Among the Irish megaliths, we observed two potential familial structures (*SI Appendix,* Fig. S10). The first is composed
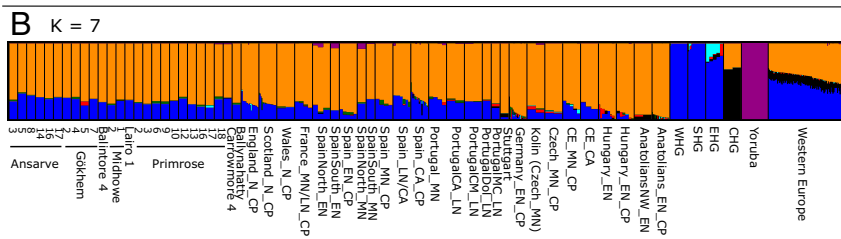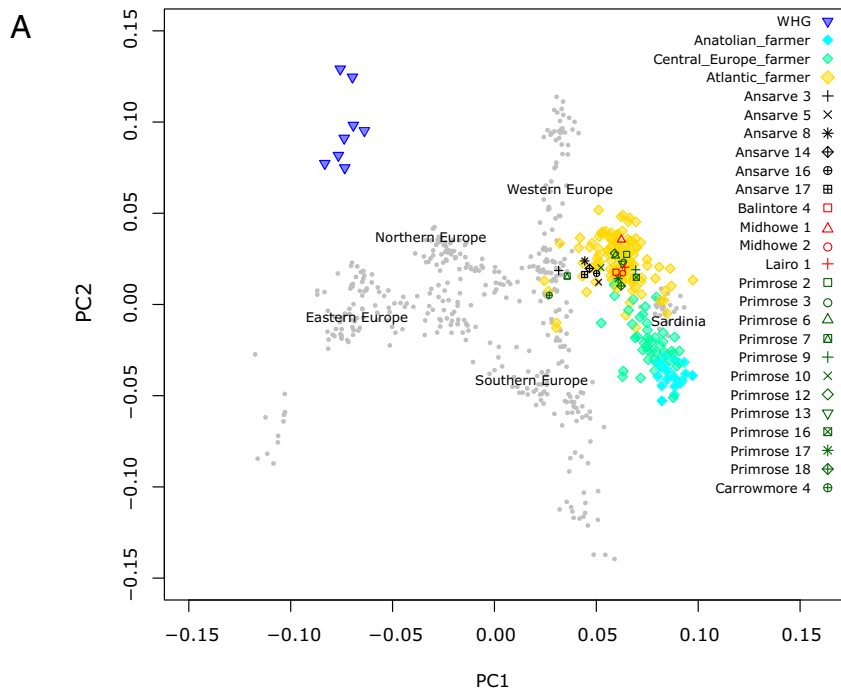
Fig. 2. (*A*) PCA of 429 present-day west Europeans (gray dots) with previously published Western HG (WHGs), Atlantic coast and Central European Neolithic farmer samples (filled symbols), and the samples from the present study (shaded symbols) projected onto the first two principal components (more details in *SI Appendix*, Section S11.1). (*B*) Inferred ancestry components (assuming seven clusters) of ancient individuals (*Methods* and *SI Appendix*, Section S11.2). All individuals to the left of Yoruba are prehistoric individuals, all of which are shotgun-sequenced unless marked with "CP" for SNP capture data. In the label names, the following letters indicate an archaeological context: CA, Chalcolithic; EN, Early Neolithic; N, Neolithic; MN, Middle Neolithic; LN, Late Neolithic. The LN individuals from Portugal come from different sites (key provided in Dataset S1.3).

of three individuals from Primrose Grange (Tomb 1; individuals Primrose 2, 17, and 18), which overlap broadly in time (Fig. 3). Primrose 2 and 17 were predicted to be related in the first degree, representing a father-daughter relationship. Primrose 17 and 18 were predicted to be second-degree relatives (harboring the same mtDNA lineage but with possibly different YDNA haplogroups) and thus could have been half-siblings or double cousins. However, the YDNA prediction is hindered by low coverage and few informative markers, and thus a grandfather-grandson or uncle-nephew relationship cannot be fully excluded.

The other Irish putative pedigree structure was integrated by two individuals from Tomb 1 (Primrose 6 and 7) and one individual from Carrowmore 4 (from the Listhogil Tomb at the Carrowmore site in close vicinity), who harbored different mtDNA lineages. While the 95% CI dating range of Primrose 6 and Carrowmore 4 overlap, Primrose 7 might be slightly younger than the other two individuals. The Carrowmore 4 and Primrose 7 males were inferred to be at least second-degree related (3.14 SE below the expected value for two unrelated individuals), and the best prediction was a first-degree relation (1.79 SE below the value for a second-degree relation, although not statistically significant at the 95% level; *SI Appendix*, section S10). If a first-degree relation is assumed, then the sole possible kin connection is a father-son relationship, because the individuals are not maternally linked. In the case of a second-degree relationship, any paternally related second-degree familial connection is possible. The other two READ-predicted second-degree kin relationships in the Irish burials (Primrose 6-Primrose 7 and Primrose 6-Carrowmore 4; 1.04 SE and 0.50 SE below the threshold for an unrelated pair, respectively) involved a familial connection of the male individuals to Primrose 6 (female).

Within the Ansarve megalith, we identify a second-degree relationship between the contemporaneous males Ansarve 14 and Ansarve

17 (Fig. 3 and *SI Appendix*, section S10). Both males have the same YDNA haplotype but different mtDNA lineages, suggesting that they could be related through any second-degree paternal kin relationship. Morphologically, Ansarve 14 was predicted to be an adult, and Ansarve 17 was predicted to be a juvenile (*SI Appendix*, section S2). Such observations might favor a grandfather-grandson or uncle-nephew relatedness over half-siblings or double cousins; however, the latter alternatives are still compatible with the data (*SI Appendix*, Fig. S12). READ analyses from other megalith burials
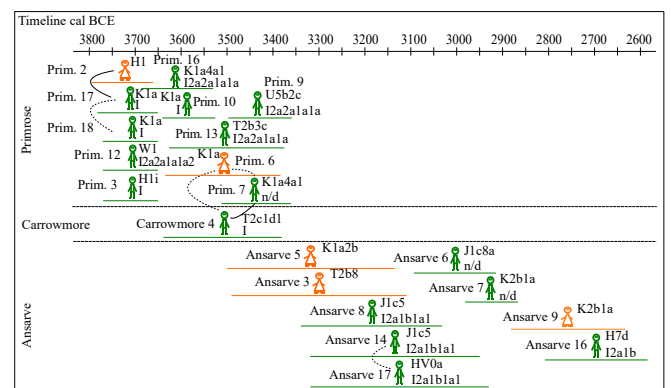

Fig. 3. Kinship relationships in the Primrose, Carrowmore, and Ansarve burials. Solid line, first degree; dashed line, second degree. Males are displayed in green; females, in orange. The MtDNA and YDNA haplogroups are presented to the right of the figures. Bars underneath figures represent calibrated dating, with 95% CI (details in Table 1 and *SI Appendix*, Table S1).

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where genetic data from at least four individuals were available per site (Gökhem, La Mina, Isbister, and Holm of Papa Westray; Fig. 1) did not reveal any evidence of genetic kinship relations. However, such observations may be hindered by the limited number of individuals investigated or by low genome coverage, which decreases the power to infer kinship (*SI Appendix*, section S10).

Discussion

The genetic variation and characteristics of individuals buried in megalithic tombs, and also from individuals buried according to other traditions, suggest that the megalithic tradition was linked to socially stratified Neolithic farmer societies, with the genetic data suggesting close connections between Neolithic populations in Atlantic Europe (5, 15, 48) (Fig. 2 and *SI Appendix*, Figs. S19–S22). Here we provide evidence of a genetic connection among Scandinavian, British, and Irish Neolithic populations. This signal is weaker than the signals observed between the Iberian Peninsula and the British Isles, however (5, 11, 15, 47, 48) (Dataset S1.3), suggesting that migration between the British Isles and Scandinavia along the Atlantic coast was less frequent than that between Iberia and the British Isles (*SI Appendix*, section S11.4).

The I2 YDNA lineages that are very common among European Mesolithic HGs (2, 3, 15, 56, 57) are distinctly different from the YDNA lineages of the European Early Neolithic farmer groups (8–10), but frequent in the farmer groups of the fourth millennium BCE (2, 3, 8–10, 15, 56, 57), suggesting a male HG admixture over time. The megalith individuals do not show higher levels of HG ancestry on the autosomes than on the X chromosome, but the Scottish_MN group shows a tendency toward a male-biased HG admixture in farmer groups, similar to previous observations (58). For the Scandinavian farmer groups, in contrast to the other megalith groups, we found an HG admixture for both the autosomes and the X chromosome. When these findings are considered together, it appears as if the social dynamics between HGs and Neolithic farmer groups, and thus the genetic admixture with HGs, differed somewhat in different geographic regions—an observation consistent with a combination of previous male sex bias admixture events occurring on the continent and more recent regional encounters with HG groups with a less pronounced sex-biased admixture.

These observations imply that the groups that erected and used the megalithic burial structures were stable and stratified, but probably not isolated farmer societies (37, 41). The genetic connection of the individuals from the Primrose Grange and Carrowmore burials, spatially distanced by only 2 km and in contemporaneous use, suggests that transgenerational patrilineal structured societies could have expanded geographically, possibly leaving a (local) genetic fingerprint related to the social dynamics of the group. Such a scenario of forming patrilineal kin groups and intergroup competition during the Neolithic could explain the inferred Y-chromosome bottleneck seen in present-day European populations (51, 59).

A central topic of discussion concerning the megalithic phenomena relates to the character of the communities that erected and used them for funerary rituals (27, 31, 37, 41, 42). The distinction of specific paternal lineages among the megaliths, a greater fraction of males than females in some megaliths, and their kindred relationships suggest that people buried in the megalithic tombs belonged to patrilineal segments of the groups/societies rather than representing a random sample from a larger Neolithic farmer community living in close vicinity. The sex ratio in the Irish megaliths is also in line with this finding. If one of the main functions of the tombs was to contain the remains of the deceased of a patrilineal segment, this would explain the inclusion of more males than females in the tombs. However, the finding that three of the five kinship relationships in these megaliths involved females indicates that female kindred members were not excluded. The observation of paternal continuity across time at the Gotlandic Ansarve megalith and at the Irish megaliths is a strong indication that specific family groups used these stone constructions for burial and other funerary practices. Of course, the patterns that we observe could be unique to the Primrose,

Carrowmore, and Ansarve burials, and future studies of other megaliths are needed to provide additional data that can inform us further about social organization in the Neolithic.

Materials and Methods

Archaeological Samples (*SI Appendix*, sections S1 and S2). Bones and teeth from human remains representing 27 individuals (Table 1) from seven sites were sampled for ancient DNA analyses; Primrose Grange (Tomb 1) and the Listhogil court cairn at Carrowmore (Ireland), the Lairo and Midhowe chambered tombs in Orkney and the Balintore short cist burial (Scotland), the Ansarve dolmen on the Island of Gotland (Sweden), and the Kolin Rondel site (the Czech Republic). Twelve samples were radiocarbon dated using accelerator mass spectrometry, and datings were available for the other samples.

Sequencing (*SI Appendix*, section S3). DNA was extracted from bones and teeth (60, 61), and DNA sequences in the extracts were converted to blunt-ended Illumina libraries. For some individuals, uracil-DNA-glycosylase (UDG)-treated, whole-genome capture-enriched, and/or single-strand libraries were also generated. All samples were prepared in dedicated ancient DNA facilities. The libraries were sequenced on Illumina HiSeq platform 2500 or XTen.

NGS Data Processing and Authentication (*SI Appendix*, section S4). Overlapping paired-end reads were trimmed and merged (62), and the fragments were mapped to the human reference genome (63). Fragments with identical start and end positions were considered PCR duplicates and collapsed into consensus sequences. Contamination was estimated based on phylogenetically informative sites on the mitochondrial genome using Contamix (64), on the X chromosome using ANGSD v.0.902 (65), and on the autosomal data using VerifyBamID v.1.1.2 (66). All libraries except the UDG-treated libraries showed signs characteristic of aDNA damage (67).

Uniparental Haplogroups (*SI Appendix*, sections S6 and S7). We inferred the most likely haplogroup from mitochondrial consensus sequence from each individual (68, 69). Y chromosomal haplogroups were further assigned by investigating informative single base substitutions obtained from the International Society of Genetic Genealogy (version 11.110 from April 21, 2016; https://isogg.org/). Geographical and temporal distribution of Y chromosomal haplogroups are outlined in *SI Appendix*, section S11.6.

Population Genetic Analysis (*SI Appendix*, section S11). The data from the investigated individuals were merged with various published datasets depending on the nature of the analyses. At each SNP position, a single read (minimum mapping and base quality of 30) was drawn at random to represent a haploid copy from the ancient individual. Transitions were coded as missing data to exclude potential postmortem damage. For each ancient individual, a PCA was conducted together with 203 modern Europeans (70, 71), and ancient individuals were plotted using Procrustes transformation (72). Ancestry components were inferred (73) based on 1,718 modern-day individuals from 179 populations and all ancient individuals (*SI Appendix*, Table S5). Common modes among the different runs were identified, and clusters were aligned across different values of K using pong (74). f3 and f4 statistics were computed (71) to estimate shared drift between populations.

Kinship Relationship Inferences (*SI Appendix*, section S10). Familial relationships were inferred (55) for individuals. Data generated with different library building strategies were handled separately to avoid potential biases.

Data Availability. Raw sequencing reads produced for this study have been deposited in the European Nucleotide Archive (accession no. PRJEB31045).

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