Genetic continuity, isolation, and gene flow in Stone Age Central and Eastern Europe

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Abstract

The genomic landscape of Stone Age Europe was shaped by multiple migratory waves and population replacements, but different regions do not all show the same patterns. To refine our understanding of the population dynamics before and after the dawn of the Neolithic, we generated and analyzed genomic sequence data from human remains of 56 individuals from the Mesolithic, Neolithic and Eneolithic across Central and Eastern Europe. We found that Mesolithic European populations formed a geographically widespread isolation-by-distance zone ranging from Central Europe to Siberia, which was already established 10,000 years ago. We also found contrasting patterns of population continuity during the Neolithic transition: people around the lower Dnipro Valley region, Ukraine, showed continuity over 4,000 years, from the Mesolithic to the end of Neolithic, in contrast to almost all other parts of Europe where population turnover drove this cultural change, including vast areas of Central Europe and around the Danube River.

INTRODUCTION

The spread of modern humans into Europe started some 50,000 – 40,000 years ago[1–3]. Before the agricultural transition that started approximately 8,500 years ago[4,5], Europe was inhabited by hunter-gatherer populations, roughly clustering into two groups as defined by archaeogenetics; Western Hunter-Gatherers (WHG) in Western Europe and East European Hunter-Gatherers (EHG)[6–8] in northeastern and in the extreme eastern frontier of Europe[9,10]. In between these core regions, the groups from the east (EHG) and from the west (WHG) probably met and admixed[11–13]. In Scandinavia, where ice coverage partially persisted until 10,000 years ago, the colonization of WHG groups took place from the south, whereas EHG groups entered from the northeast, likely following the Norwegian Atlantic coast from the north to the south[11], creating an admixture pattern that goes in the opposite direction to central/eastern Europe. However, our knowledge concerning the history and dynamics as well as the time scale of genetic admixture and continuity of the Mesolithic populations across Europe is limited.

The population structure of Stone Age Europe experienced a significant change in the early Holocene. This change was associated with the spread of farming groups from the Near East brought by migrating people (European Neolithic, EN)[14–16], which were genetically closely related to the groups from the Neolithic Anatolia (AN)[17–19] and more distantly to the hunter-gatherers from the Caucasus region also known as CHG[20]. The mode and level of population interaction in the initial and subsequent times of the European Neolithic farmers and hunter-gatherers has been a matter of debate for very long time. The current consensus points to geographically and temporally varying level of genetic admixture of the EN and WHG groups[7,21–24] starting already at the early stages of the arrival of the former in central Europe[24]. Based on evidence from the archeological record, there may have been differences in the levels of cultural contacts between the farmer and hunter-gatherer groups in a west-east gradient of the widely spread Early Neolithic Central European Linear Pottery culture (LBK)[25]. However, the suggested interactions may have been in form of exchange of goods rather than genetic admixture.

In addition to the variable contacts and interactions between the hunter-gather and incoming farmer groups, in some European regions (for instance in parts of Scandinavia, the Baltic region, and the Eastern Europe) the hunter-gathering lifeway prevailed for much longer in comparison with the Southern and Western Europe. In Ukraine for example, the steppe and forest steppe zones of the North Pontic region were inhabited by hunter-gatherer communities still during the Neolithic sustaining mostly on aquatic resources[26]. A similar type of development took place in these communities as in the Neolithic farming groups. For instance, in some parts of Eastern and Northeastern Europe pottery was introduced but mainly hunter-gatherer subsistence patterns prevailed[27,28]. Genetic data from some of these groups have shown that the genetic makeup before and after the European agricultural dawn remained similar in contrast to Central and Western Europe[12,29,30].

To improve our understanding of the level, character and regional variability of contacts between the Central and Eastern European Stone Age groups, we sequenced and analyzed whole genomes of individuals who lived before and after the Neolithic transition (i.e., 7,500-5,500 BP) in the eastern frontier of Europe. The investigated area encompasses an area covering modern-day Romania, Poland, and the lower Dnipro Valley region in Ukraine over a time span of approximately 5,000 years (ca. 10,500-5500 BP).

RESULTS & DISCUSSION

To investigate the genetic affinities in Stone Age Central and East Europeans, we generated genome-wide sequencing data from a collection of 56 individuals from Epipalaeolithic/Mesolithic, Neolithic and Eneolithic Poland, Romania & Ukraine (Fig. 1A & B; see Supplementary Information and Dataset S1 & S2). The depth of coverage per individual ranged from 0.01 to 4.55 X.

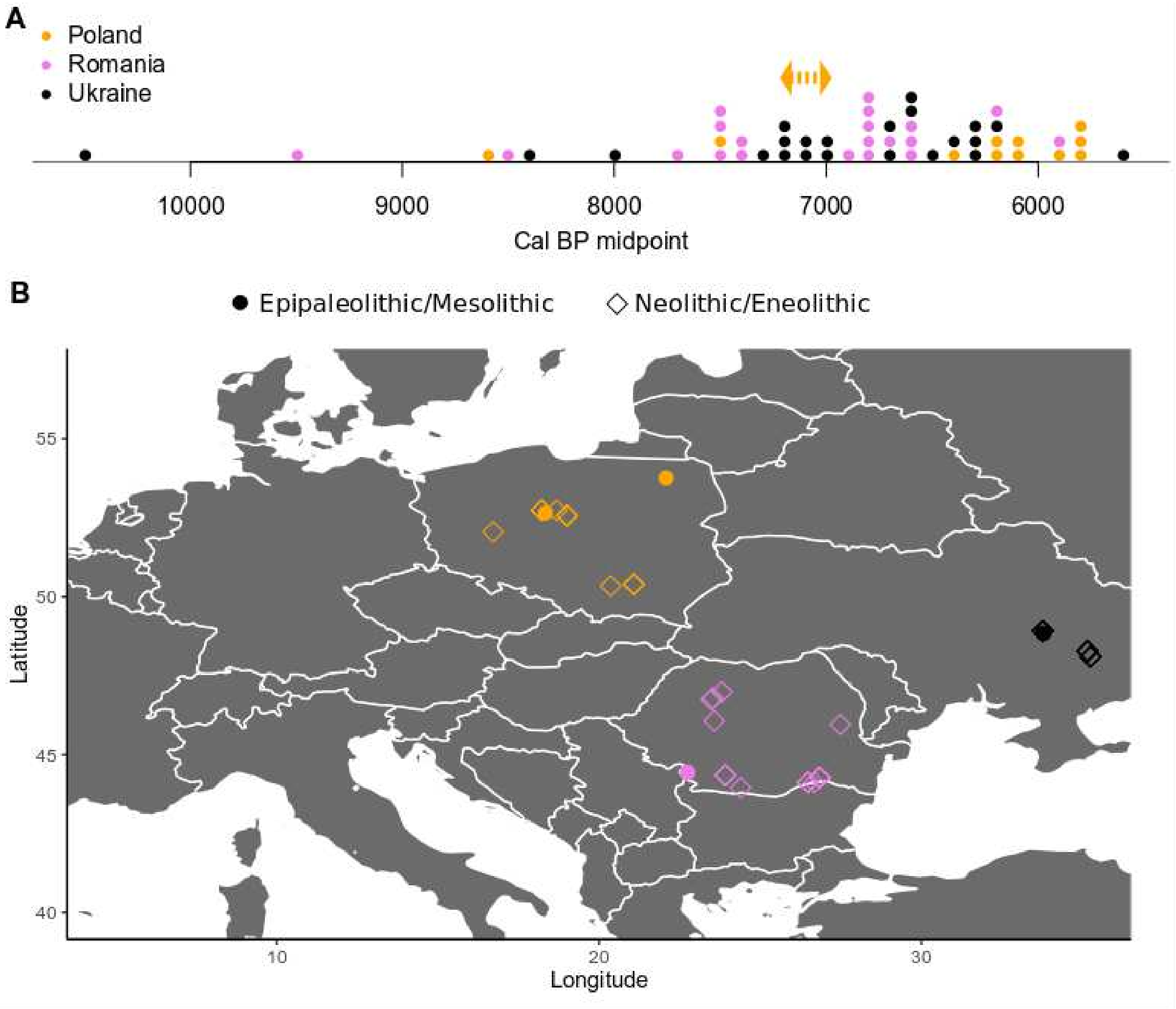

Figure 1. Summary of the newly analyzed individuals in this study. (**A**) The distribution of cal BP median. The orange arrow shows the context-based approximate age of three LBK samples (lbk101, lbk102 and lbk104). (**B**) The geographic location of the newly sequenced individuals.

Over 4,000 years of genetic continuity in the Stone Age lower Dnipro Valley region in Ukraine

To characterize the genetic structure of our data, we first used a principal component analysis (PCA) for the dataset together with a collection of Stone Age and Bronze Age individuals across West Eurasia (Supplementary Dataset S3). The PCA placed all the Epipaleolithic/Mesolithic Central and East European individuals on a cline between WHGs (represented by individuals Bichon, Loschbour, Ranchot88, Rochedane, and Villabruna[6,8,20]) and the Upper Paleolithic Afontova Gora3[8] (Fig. 2A), consistent with previous findings[10,12]. Comparative Mesolithic hunter-gatherers from Western Russia (EHG & WRuHG), the Baltic region (BHG), and Sweden & Norway (SHG) also fell within this cline. To gain further insight into the genetic composition of the studied groups, we inferred ancestry components[31] (Fig. 2B), including a broader set of comparative individuals from the Stone Age and from modern times, sampled across Eurasia (Supplementary Information & Supplementary Dataset S3). The individuals from the Neolithic lower Dnipro Valley were genetically very similar to the Epipaleolithic/Mesolithic individuals from this region. In contrast, the Neolithic/Eneolithic individuals from the Romanian and Polish sampling sites displayed the same ancestry components as other European farming groups, and were genetically similar to the Anatolian Neolithic farmers[17–19]. These results were also supported by the patterns of allele sharing with WHG and EN (Fig. 3A-C) as well as the uniparental markers (Supplementary Dataset S1, S4 & S5).

To test for genetic continuity in the three regions investigated in this study, we utilized the f_3-outgroup test f_3(Yoruba; X, Y), where X was the test individual and Y the highest coverage Mesolithic individual from the same region. The f_3-test verified (Fig. 3F) that the populations of the lower Dnipro Valley region stayed genetically similar from the

Mesolithic to the Neolithic. The difference in the fresh water reservoir effect corrected cal BP median age estimates between the oldest and the youngest individuals (ukr125: 10,547 cal BP, ukr123 6,233 cal BP; Dataset S1) indicated that the genetic continuity in this region lasted more than 4,000 years. In contrast, for the Romanian and Polish individuals, there is a distinct genetic discontinuity between the pre-Neolithic and the Neolithic individuals, indicating higher levels of gene flow (Fig. 3D-E).

Finally, from the genome sequence data, we can assess genetic diversity (conditional nucleotide diversity[32]), which gives indications on past population sizes. The conditional nucleotide diversity was very similar for the Mesolithic and Neolithic populations from the Dnipro Valley region, in contrast to Romania and Poland where the diversity is much higher among the Neolithic individuals in comparison with any Mesolithic pairs (Fig. 4C). Hence, we concluded that the Dnipro Valley population likely stayed relatively stable in size (at least in terms of effective population size) and was unaffected by admixture with European Neolithic farmers/Anatolian farmers.

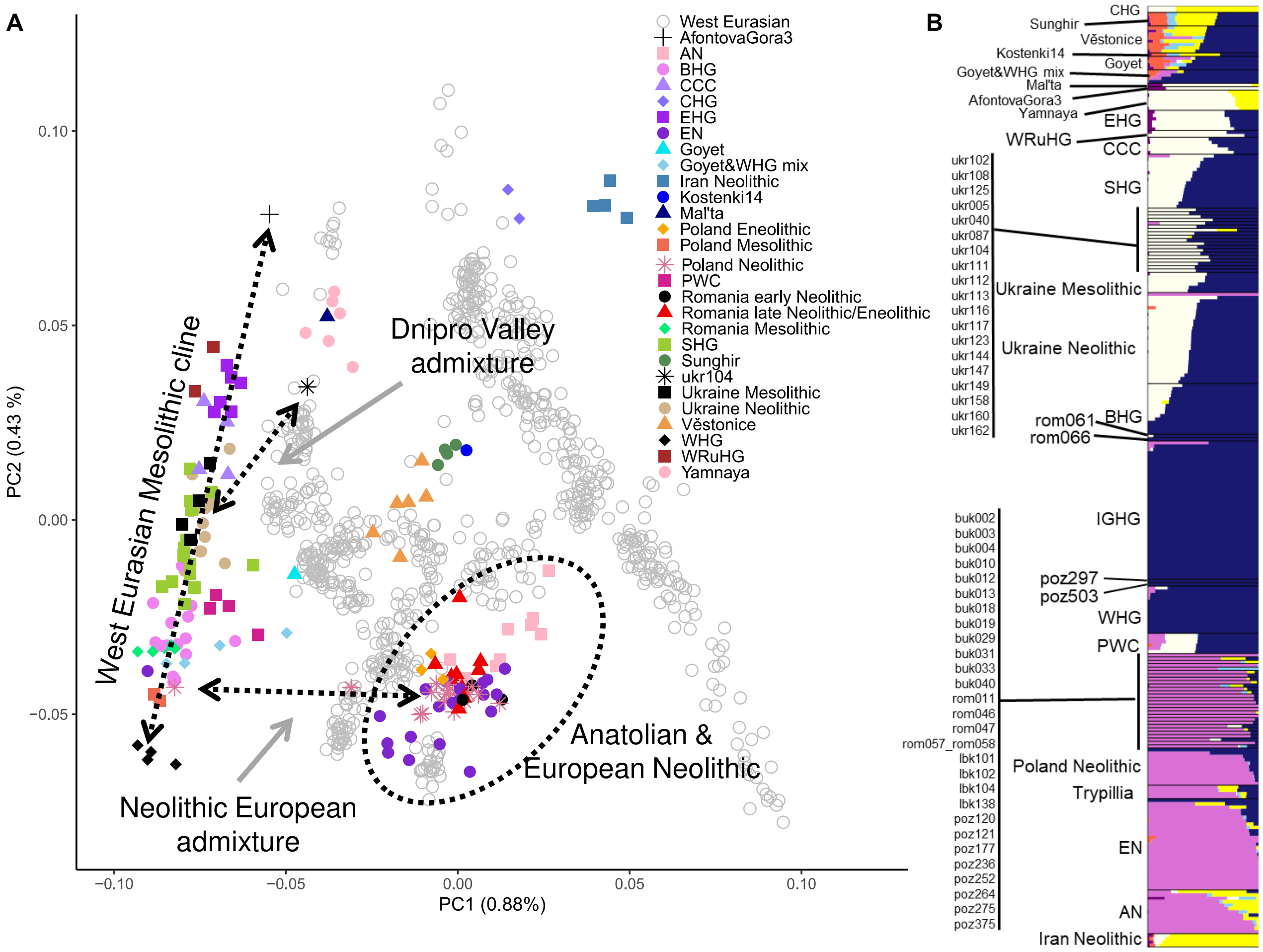

Figure 2 Summary of the population structure of Stone Age Europeans. (**A**) Principal component bi-plot of selected Paleolithic, Mesolithic, and Neolithic West Eurasian individuals projected onto eigenvector space estimated from a set of modern-day West Eurasian groups from the Human Origins dataset[6]. Only individuals of which have at least 10 000 called SNPs are shown on the plot. Arrows and the black circle highlight the groups including individuals investigated in this study. Full ancient individual annotation is available from Supplementary Fig. S5:1 (**B**) Admixture plot showing the representative run of K = 7 and admixture proportions estimated for the ancient individuals. The sample names are shown for individuals from this study. The full Admixture plot is available from Supplementary Fig. S5:2. Abbreviations: AN = Anatolian Neolithic, BHG = Baltic Hunter-Gatherers, CCC = Comb Ceramic Culture from the Baltics, CHG = Caucasus Hunter-Gatherers, EN = European Neolithic, IGHG = Iron Gates Hunter-Gatherers, PWC = Pitted Ware Culture from the Scandinavian Peninsula, SHG = Scandinavian Mesolithic Hunter-Gatherers, WHG = Western Hunter-Gatherers, WRuHG = West Russian Hunter-Gatherers.

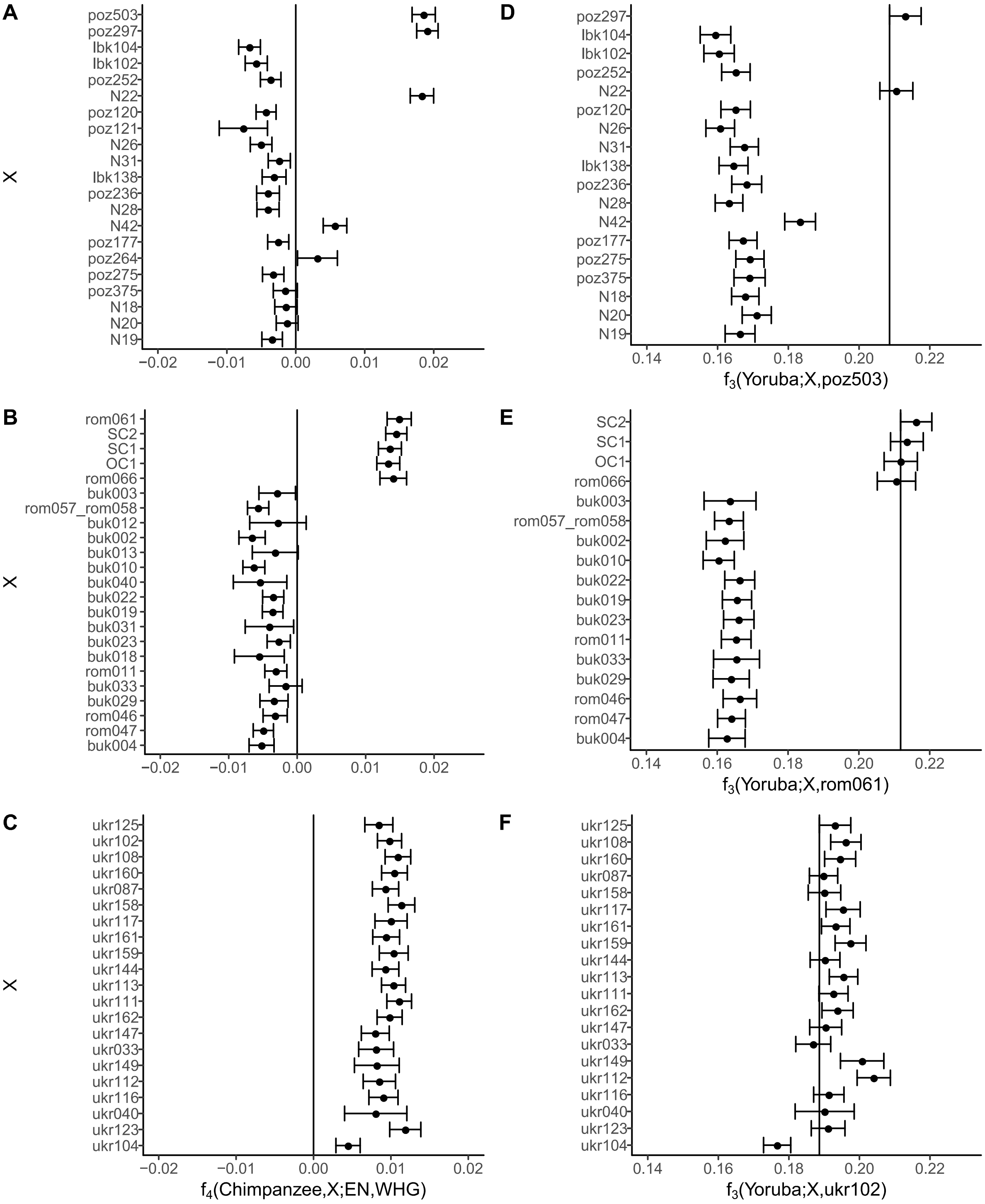

Figure 3. Patterns of allele sharing in Mesolithic, Neolithic and Eneolithic Central and Eastern Europeans. (A-C) f_4-statistics testing allele sharing between Mesolithic Central European hunter-gatherers (WHG, Loschbour) and European Neolithic farmers (EN, LBK) (D-F) Regional continuity f_3-outgroup test. The vertical line shows the lower point of the 95% confidence interval for the comparison with the oldest dated individual. The individuals included were excavated in modern-day Poland (A & D), Romania (B & E), and Ukraine (C & F). The data are shown for newly produced data and, additionally, for three Mesolithic Romanian (OC & SC) and eight Neolithic individuals from Poland (N) previously published in González-Fortes et al. (2017) and Fernandes et al. (2018). Error bars indicate the 95 % confidence intervals from block Jackknife standard errors. The individuals were ordered based on their cal C14 age. All the statistics were calculated using the 1000 genomes transversion overlap panel. Only tests which are based on at least 10 000 (f_4) and 500 (f_3) sites are shown.

Isolation-by-distance in Mesolithic Western Eurasia

To further investigate the potential admixture between the Upper Paleolithic Siberian group and WHG, we first tested if Loschbour (representing WHG) forms a clade with the European Mesolithic individuals from the admixture zone using f_4-test (chimp, AfontovaGora3; X, Loschbour) using the Human Origins overlap panel. The f_4-values were negative for all, but for the Polish individuals, they were not significantly different from 0 (Supplementary Fig. S5:3A). To increase the power of the test, we calculated f_4(Yoruba, EHG; X, WHG) from the 1000 genomes overlap panel and confirmed the significant contribution from the eastern lineage to all Central and East as well as North European Mesolithic individuals investigated (Supplementary Fig. S5:3B), where Sidelkino[9] represents the EHG and Loschbour[6] the WHG. A model-based two-source analysis separated the admixture model (WHG-AfontovaGora3) from the single source models in 15 cases. The estimated admixture proportions of WHG-related ancestry ranged from 50.9 % (40.9 % - 60.9 %, 95 % Jackknife CI) for Sidelkino to 83.7 % (73.9 % - 93.5 %) for ZVEJ25 (Supplementary Dataset S6).

The different admixture models between the Paleo Siberian-WHG gradient were also tested (using qpGraph[33]) including representative groups from the gradient. The stepping stone like graph (Fig. 5A) including admixture from a group related to the Paleolithic Siberian (represented by AfontovaGora3) in EHG (represented by Sidelkino) and this lineage further re-admixing with the WHG lineage was consistent with the data (worst Z-score 0.978, f_4(Sidelkino, Loschbour; ukr102, ble008)). Furthermore, as three other tested models without this admixture were inconsistent with the data (Supplementary Fig. S5:4), the admixture between the West European and Siberian lineages were further strengthened. The connection between the EHG and the Paleolithic Siberian lineage has been reported also in[8], but it was not clear that EHG is part of the Paleo Siberian-WHG gradient previously.

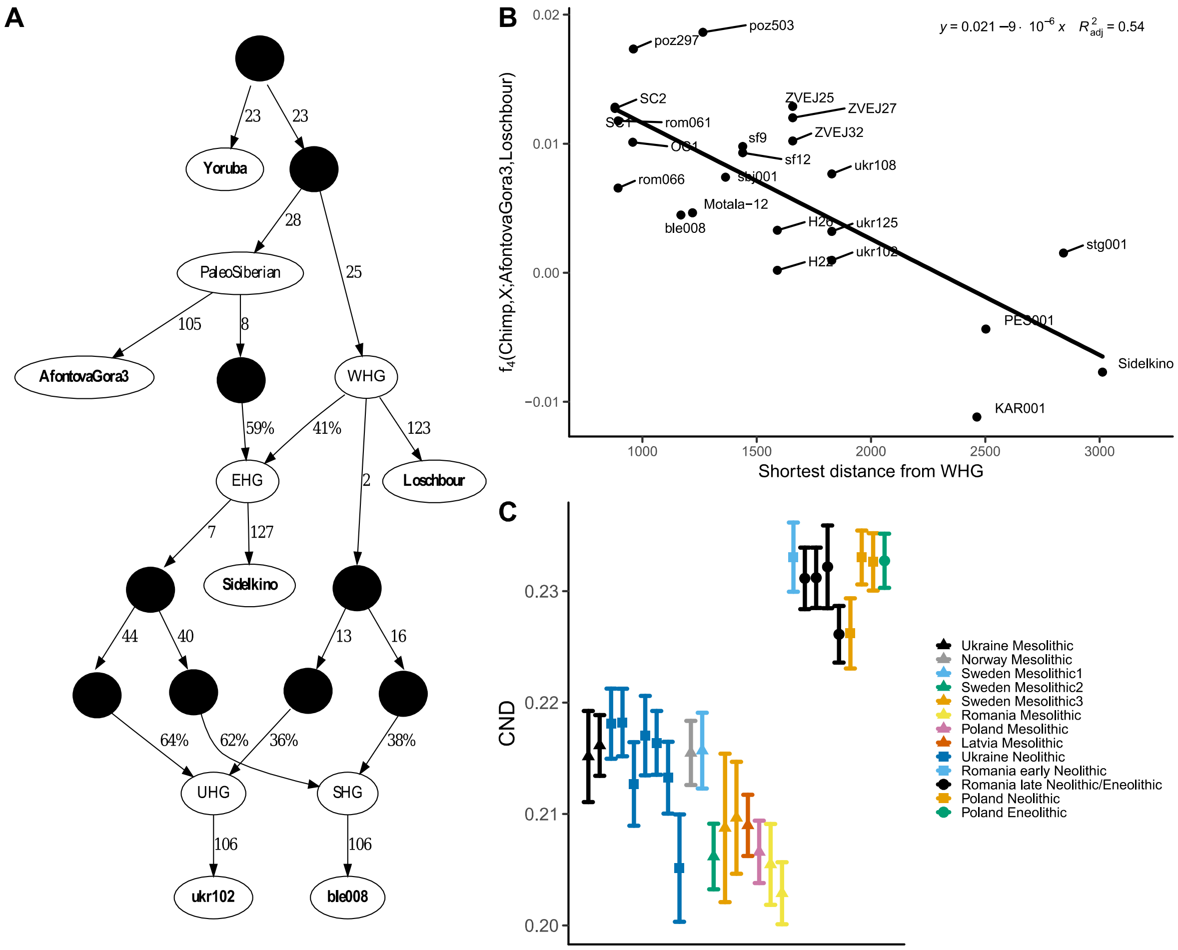

Figure 4. Patterns of admixture and genetic diversity in Stone Age Europeans. (**A**) qpGraph model including stepwise admixture between Paleo Siberian (PaleoSib, represented by AfotovaGora3), Western Hunter-Gatherers (WHG, represented by Loschbour), and Eastern Hunter-Gatherers (EHG, represented by Sidelkino). The additional admixture nodes included here were the Ukrainian Mesolithic (ukr102) and Scandinavian Mesolithic (ble008) individuals. The data-point nodes are in bold. (**B**) Scatterplot and linear regression model of distance from the closest WHG data-point and allele sharing (f_4) between WHG (Loschbour) and Paleo Siberians (AfontovaGora3). (**C**) Conditional nucleotide diversity for selected Mesolithic, Neolithic and Eneolithic European individuals. The individual pairs included in this analysis are available at Supplementary Dataset S7. The Mesolithic individuals from the region of Sweden were split into three groups Sweden Mesolithic1: two individuals from Huseby Klev[34]; Sweden Mesolithic2: two individuals from Gotland[11]; Sweden Mesolithic3: four individuals from Motala[6].

The patterns of genetic admixture in the Mesolithic of the European continent suggest a geographical dependency in the Paleolithic Siberian-WHG ancestry proportions. Previous archaeogenetic analysis has indicated that the Eastern and Western Hunter-Gatherer lineages were admixed in Scandinavia forming a EHG/WHG gradient in Northern Europe

[11]. We tested the fit of the isolation-by-distance admixture model (admixture IBD) in the Paleo Siberian-WHG cline using a linear regression analysis of the level of allele sharing (f_4-test) and distance from the WHG core region. As a measure of the distance from the WHG core region, we took the shortest optimal topology aware route from five WHG points (Supplementary methods and Supplementary Fig. S5:5). The linear regression analysis indicated a significantly decreasing proportion of the WHG ancestry and increasing Paleolithic Siberian (represented by AfontovaGora3) ancestry in West Eurasia as a function of minimum distance from the WHG core region (linear regression coefficient for minimum distance = -9.0×10^{-6}, SE = 1.7×10^{-6}, t-value = -5.3, p-value = 2.5×10^{-5}; Fig. 4B; Supplementary Fig. S5:6). The results were significant also after removing the possible leverage points from the analysis (linear regression coefficient for minimum distance = -6.5×10^{-6}, SE = 3.0×10^{-6}, t-value = -2.1, p-value = 0.048; Supplementary Fig. S5:7-8).

Gene flow between two genetically differentiated populations is also expected to increase genetic diversity as previously observed in Scandinavia[11]. The highest diversity is expected when the ancestry proportions are close to equal given other population processes being equal. To test this, we calculated conditional nucleotide diversity and found that the diversity among the Mesolithic pairs was in line with the expected increase in diversity as a function of admixture proportions (Fig 4C). Taken together, the expectations of the IBD admixture model indicate long-distance, stepping-stone-like, gene-flow between Europe and Siberia in pre-Neolithic Europe.

Gene flow to the lower Dnipro Valley population

Even though the major ancestry components of the Mesolithic and Neolithic lower Dnipro Valley population derived from WHG and Paleolithic Siberian lineages (where EHG likely functioned as a stepping stone), we also found that a three-way population admixture model (EHG-WHG-CHG) fits the genetic ancestry composition of this population (Supplementary Dataset S8). We estimated that approximately 7.4 % (0.15 % - 14.7 %, Jackknife 95 % CI) of the genetic ancestry in the Dnipro Valley population is derived from a CHG population indicating a genetic connection between the Caucasus and the North Pontic region in the Mesolithic/Neolithic. The allele sharing with CHG was significantly higher among the Neolithic Dnipro Valley individuals (Supplementary Dataset S9) which means that at least some level of this ancestry sharing is due to mixing during the Neolithic.

In addition, the Eneolithic individual from the lower Dnipro Valley region (Deriivka II cemetery) archeologically classified as Serednjostogivs'ka (Sredny Stog) horse keepers (ukr104, c. 5,650-5,477 cal BP) showed smaller level of allele sharing with other individuals from the same region (Fig. 3F). This indicates gene flow from a population that is genetically differentiated from the preceding local population. This individual (ukr104) was genetically similar to the Bronze Age Yamnaya individuals from Samara, the CHG and the Neolithic Iranian (Fig. 2A-B). To test this possible gene-flow, we modeled ukr104 as a mixture of a set of lower Dnipro Valley individuals (ukr087, ukr102, ukr111, ukr113, ukr160) and Yamnaya[35] using qpAdm[33]. Other ancient neighboring groups AN, CHG, EHG, Neolithic Iranian WC1, Mal'ta, WHG and Sunghir were used as 'right' populations in addition to an outgroup (chimp, Supplementary Dataset S10). The admixture model fitted the data well ($\chi 2 = 2.37$, tail probability = 0.88, df = 6), while the single-source models were rejected (tail probability < 0.05, Supplementary Dataset S10). The estimated admixture proportions were 33.2 % (25.0 % - 41.4 %, 95 % Jackknife CI) of the local Meso-Neolithic Dnipro Valley ancestry and 66.8 % (58.6 % - 75.0 %) of the Yamnaya related ancestry.

Admixture through time in the Neolithic Central and Eastern Europe

To explore the admixture between the Neolithic East European and the descendants of European Mesolithic hunter-gatherer groups, we tested if the hunter-gatherers from Poland and Romania (poz297 and rom061, respectively) share more alleles with the Romanian and Polish Neolithic/Eneolithic individuals when compared with early Neolithic Central Europeans. In comparison with the early Neolithic LBK individual from Germany[6], a significant increase in allele sharing with the local hunter-gatherers were detected in 16 out of 30 newly produced Neolithic/Eneolithic individuals from Poland and Romania (Supplementary Dataset S11).

The estimated ancestry deriving from the local Mesolithic hunter-gatherers (Z-score > 2, f_4-ratio test) ranged from 9 to 20 % in the Romanian Neolithic/Eneolithic individuals while it was 9 - 97 % among the Neolithic/Eneolithic individuals from Poland (Supplementary Dataset S11). We also observed a significant increase in the proportion of admixture through time (linear regression coefficient for ^{14}C median = -4.8×10^{-5}, SE = 1.8×10^{-5}, t-value = -2.7, p-value = 0.012; N22, N42 and poz264 excluded with the most extreme α values). This resurgence of the local Mesolithic ancestry in the Eneolithic has also been found in previous studies in other parts of Europe[23,36].

Kinship in Stone Age Europe

The patterns of genetic kinship in pre-historic societies can inform us about their social organization. Hence, we also investigated close kinship (1st and 2nd degree kin relations) among the studied individuals within population using the READ software package[37]. We detected two kin trios (standard error scaled distance normalized mean P0 score > 1.96) among the newly sequenced individuals (Supplementary Dataset S12). The first trio from the Boian context from Curătești (Romania) included two adult female individuals and one adult male (buk019, buk022, buk023, hereafter Curătești family). The second trio of adult males was found among the individuals from Yasinovatka, Ukraine (ukr159, ukr160, ukr161, hereafter Yasinovatka family) (Fig. 5A & D). All data for the detected kin were derived from single bone specimens and single extracts for each individual.

From the Curătești family, buk019 and buk023 were first-degree relatives, while buk019 and buk023 were second-degree relatives to buk022 (Supplementary Dataset S12). All three carried mt haplogroup K1a+195 (Supplementary Dataset S1 & S4) suggesting that they were possibly maternally related (Fig. 5A-C). Assuming that the shared uniparental haplogroups indicated direct matri- and patrilineality, we constructed possible genealogies for the detected families. The kinship assignments are consistent with the genealogical models where buk022 was a grandmother or an aunt of the siblings buk019 and buk023 from their mother's side. Equally possible models are that buk022 was a niece of the siblings buk019 and buk023 from their sister's side, buk022 was a maternal half-sib of the full-sibs buk019 and buk023, or buk22 was a double-cousin of the full-sibs buk019 and buk023 or, alternatively, buk022 was a double-cousin of buk023 and niece of buk019, and buk023 was a mother of buk019 (Fig. 5B & C). The radiocarbon inferred age estimates overlapped for all three individuals (Supplementary Dataset S1).

The Yasinovatka family members were all males and two of them were found from the same pit (Supplementary information). We found that ukr160 and ukr161 were second-degree relatives, while ukr159 was a first-degree relative of both ukr160 and ukr161 (Supplementary Dataset S12). Two individuals in this trio (ukr159 & ukr160) had U4b1a mt haplogroup, and the third had T2a1b (Fig. 5A; Supplementary Datasets S1 & S4) indicating a non-maternal relationship between the first two and ukr161. The Yasinovatka family members' Y-haplogroups fell within the I clade (Fig. 5D; Supplementary Dataset S1

& S5), suggesting a possible patrilineal relationship. The difference in Y-haplogroup assignment precision likely explains the difference in the final haplogroup assignments (Fig. 5D) since no data were available on the I2a2 defining mutations for the low coverage ukr159 (Supplementary Dataset S5). Despite the occasional difference in the called Y genotypes, we concluded that I was the most likely Y haplogroup for all of the Yasinovatka family members (Supplementary Dataset S5 & S13). These results are compatible with a model where ukr159 and uk160 were brothers, and ukr161 was the son of ukr159 (Fig. 5E). Based on the ^{14}C, ukr160 likely died slightly earlier (Fig. 5D) than the other two Yasinovatka family members.

An additional kin pair was detected among the previously published Stone Age Ukrainian Dnipro Valley individuals[12] and the dataset from this study (Supplementary Dataset S12). This pair was the first-degree kin from Mesolithic Deriivka I (ukr102 from this study & I5876 from Mathieson et al. 2018[12]). Both analyzed individuals were males who carried the same mt & Y haplogroups (Supplementary Dataset S1, S4 & S5). These findings are in line with the genealogy where these two individuals were brothers even though we cannot rule out the parent-offspring kinship, if they carried the same mt haplogroup by chance.

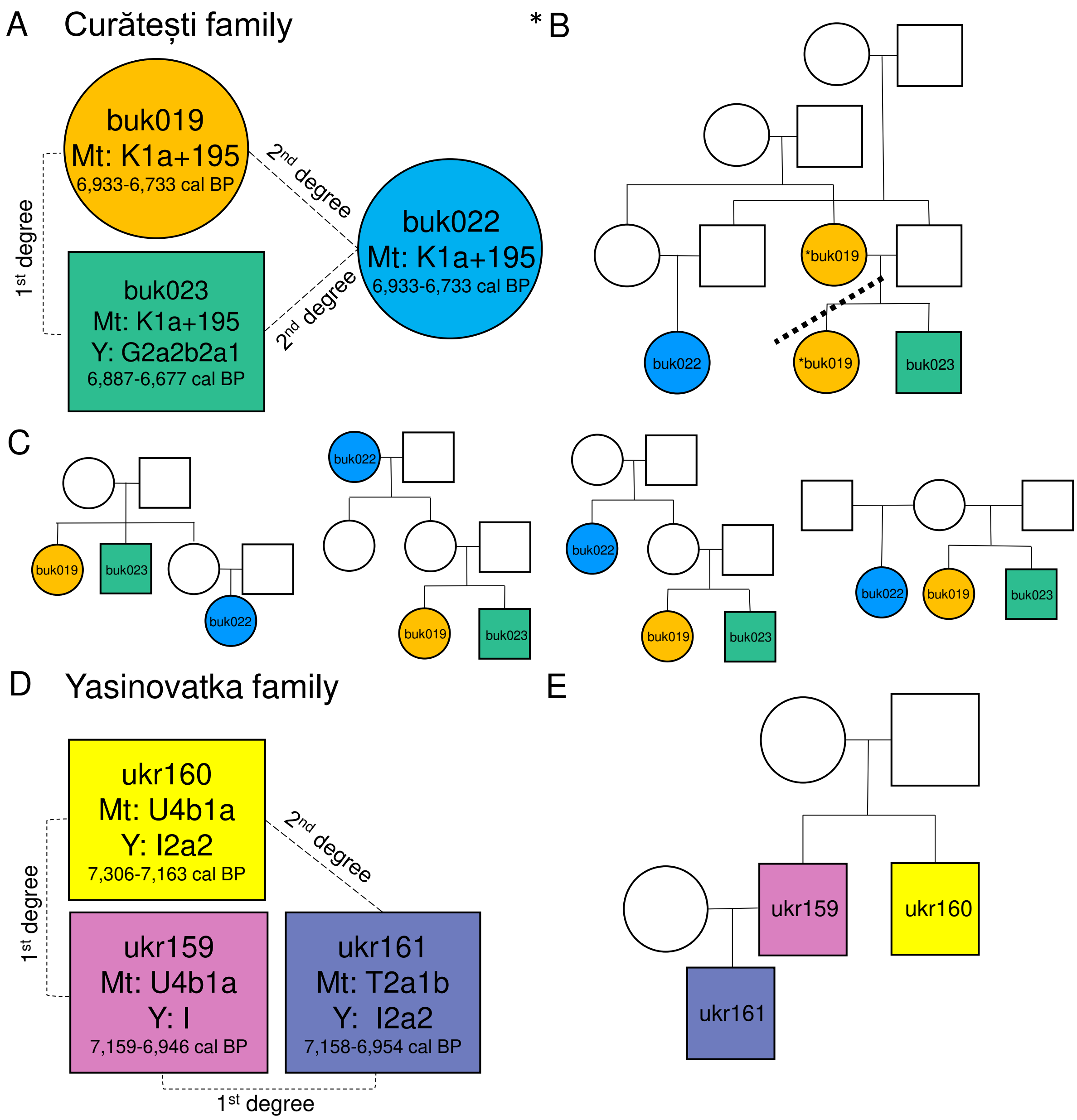

Figure 5 Summary information for the detected family trios from Neolithic Ukraine and Romania. (**A**) Information on the Curătești family members. (**B & C**) Six possible genealogical models of the Curătești family. *Two very similar double cousin scenarios where buk019 is either the mother or sister of buk023 are depicted on the same figure (B) where the dashed line separates the two alternatives. (**D**) Information on the Yasinovatka family members. (**E**) Suggested genealogy of the Yasinovatka family.

Among the individuals from Poland, we did not find any first- or second-degree kin pairs (Supplementary Dataset S12). Interestingly, two of the samples from the Krusza Zamkowa 3 cemetery were buried in close proximity, which has earlier been suggested to indicate their biological relatedness[38]. Similar to the results from Juras et al (2017)[39], we can conclude that these two individuals were not genetically related, at least not in the form of full siblings, mother-daughter, aunt-niece or grandmother-granddaughter. These individuals were also not related to the adult female lbk138 buried approximately 25 meters away. These burials are exceptionally richly equipped, with similar types of beads and adornments and have even been designated as "princess graves"[40]. A lack of maternal kinship among individuals buried close to each other have previously been found among LBK in Karsdorf in Germany [41]. Thus, social ties rather than genetic kinship - may have been of importance in burial arrangements in the Krusza Zamkowa community[42]. Different non-biological relations among individuals in pre-historic burials have recently been discussed[43]. It has also been hypothesized that other factors, related to socioeconomic organization possibly linked to specific activities, may have played a role for burial practices[44,45].

Conclusions

In this study, we have investigated the genetic landscape of Central and Eastern Europe before and after the European Neolithic expansion. One of the most striking findings was that before the dawn of the European Neolithic, Central and Eastern Europe was inhabited by a population that descends from a gradient admixture population between genetically distinct West European and Siberian hunter-gatherer groups. Such a pattern suggests long distance population genetic connectivity, likely via a 'stepping-stone' admixture model. The genetic descendants of these Mesolithic populations were in many areas assimilated or replaced by incoming farmers during the Neolithic, and the 'Mesolithic' populations remained dominant only in the East and Northeast European frontier and some geographical regions in Southern Scandinavia. In the lower Dnipro Valley region in Ukraine, the direct descendants of the Mesolithic population continued being the dominant group for thousands of years after the start of the European Neolithization, and the end of this continuity was associated with the Eneolithic/Bronze Age migration wave from the

East. Hence, we conclude that the Dnipro Valley region's Neolithic cultural innovations, such as adoption of pottery (further from pointed-bottom vessels to flat bottomed ones), pioneer animal husbandry (cattle, pig, sheep & goat, agriculture e.g., barley)[46] and the changes from contracted to extended supine burials were not associated with gene flow from Anatolia, as was the case for most the regions located further west.

Our analysis of close genetic relatedness, on the one hand, revealed the role of genetic relatedness in burial practices in cultures across Mesolithic, Neolithic and Eneolithic Europe. One the other hand, the results also pointed to a possibility of non-genetic connections such as in the Neolithic Late Lengyel culture Kruza Zamkova case exemplified here. These observations, together with previous investigations of close kin relations in the Stone Age[47–50], suggest a variety of different views and practices of biological and potentially non-biological kin relations.

METHODS

Sampling & data production

Bone and tooth material from 56 Mesolithic, Neolithic and Eneolithic individuals from Poland, Romania and Ukraine were collected for the purpose of this study. The samples were radiocarbon dated either in Beta Analytic Carbon Dating Service in Florida, USA or in Poznań Radiocarbon Laboratory in Poland, or previously published dates were collected. The final dates were calibrated using Oxcal v4.4.4, IntCal 20 and freshwater reservoir effect (FRE) correction was applied sample specifically depending on the stable isotope-based dietary analysis.

We extracted DNA of these samples and built blunt-end and Uracil-DNA Glycosylase treated DNA libraries at dedicated ancient DNA laboratories at Uppsala University, Sweden or at Adam Mickiewicz University in Poznan, Poland. The built libraries were sequenced at SciLifes SNP & SEQ Technology platform in Uppsala, Sweden, using either Illumina HiSeq 2500 or HiSeq X Ten system with paired-end chemistry.

Sequence data processing, quality control, and summary statistics

From the obtained raw sequence data, the adapter sequences were first trimmed and the overlapping reads were merged using either AdapterRemoval v. 2.1.7[51] or MergeReadsFastQ_cc.py[52]. Next, the read were aligned to the human reference genome version hs37d5 using bwa aln[53]. After alignment highly divergent and short reads were removed, duplicates were removed, and summary statistics of the dataset were calculated using an in-house pipeline described in detail previously[11,14,32,49].

For each sample, we visually verified the post-mortem damage at the fragment ends of at least one successful blunt-end library using MapDamage v.2.0.8[54]. The genetic sex of each individual was determined based on the sex chromosome read ratios[55,56]. We called mitochondrial haplogroups using a combination of HaploGrep v. 2.1.16[57] and online version of HaploFind[58] from mitochondrial consensus sequences generated using ANGSD v.0.921[59]. The haplogroups of the Y chromosome we called using an in-house SNP calling and Y haplogroup classification pipeline that is based on ISOGG SNPs[49].

Contamination estimates were calculated from mitochondrial (all samples) and X chromosome (male samples) datasets using methods described in[59–62].

Comparative datasets and SNP call

For genome-wide analyses, we called pseudo-haploid genotypes from the newly generated and comparative ancient samples[6,8,20–22,29,30,32,34–36,49,9,50,63–69,10–12,14,17–19] (see Supplementary Dataset S3), and overlapped them with reference genotype panels using an in-house pipeline first described in[14]. As a reference panels we used the Human Origins dataset and a set of transversion SNPs from the 1000 genomes SNP dataset that had at least 0.1 minor allele frequency among the Yorubas[11,70].

Population genetic analyses

We performed principal component analysis (PCA) using EIGENSOFT smartpca[71,72] and population model-based estimation of ancestry proportion estimation using the software packages ADMIXTURE[31]. West Eurasian, Central Asian & Siberian groups from the Human Origins dataset were used as a modern reference. In the principal component analysis, the ancient samples were projected onto the PC space estimated from the modern West Eurasian populations.

Three and four population f-test were used to test the level of shared drift (aka f_3-outgroup test) and allele sharing[33]. These tests were performed using AdmixTools v. 20160803[33] wrapped in an R[73] package admixr v. 0.7.1[74]. Admixture models and admixture graphs were tested using qpAdm v. 401 & qpGraph v. 6100 from the AdmixTools package.

To detect close genetic kin among the individuals studied we used the software package READ[37]. The kinship analysis was run regionally within groups to avoid population structure to affect the kin estimation.

Isolation-by-distance and admixture through time analyses

We used linear regression analysis to study the relationship of genetic allele sharing with Paleo Siberian and WHG cluster (measured as f_4-test), and landscape aware shortest distance estimates between datapoints and the WHG core region[6,8,20]. The minimum accumulative travel cost from the WHG core region to the admixed Mesolithic European sites were estimated using path distance and least-cost path computation taking into account topology and water content of each cell characteristics. Current day topology and land use were used as a proxy for the Mesolithic values.

To study the relationship between Neolithic hunter-gatherer admixture in the farming groups through time, we calculated the proportion of hunter-gatherer admixture for each individual using f_4-ratio test[33] where the local hunter-gatherer individuals were used as an A and B groups, Anatolian Neolithic Bar8[17] as a C and chimp as an outgroup. The calibrated age midpoints were used as a measure of the sample age.

All linear regression analyses were performed in R using the function lm. The lm diagnostic plots were visually inspected to evaluate the fit of the model assumptions.

Data availability

The sequence data used in this study will be available from European Nucleotide Archive under the accession numbers ENA#####-ENA###### upon publication of the study.

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