Report

Current Biology

The Neolithic Transition in the Baltic Was Not Driven by Admixture with Early European Farmers

Highlights

- A degree of genetic continuity from the Mesolithic to the Neolithic in the Baltic
- Steppe-related genetic influences found in the Baltic during the Neolithic
- No Anatolian farmer-related genetic admixture in Neolithic Baltic samples
- Steppe ancestry in Latvia at the time of the emergence of Balto-Slavic languages

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In Brief

Jones et al. present genome-wide data spanning the Mesolithic-Neolithic transition in Latvia and Ukraine that show that massive migration of Anatolian farmers was not a universal driver for the spread of Neolithic lifeways and possibly Indo-European languages throughout Europe.

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The Neolithic Transition in the Baltic Was Not Driven by Admixture with Early European Farmers

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SUMMARY

The Neolithic transition was a dynamic time in European prehistory of cultural, social, and technological change. Although this period has been well explored in central Europe using ancient nuclear DNA [1, 2], its genetic impact on northern and eastern parts of this continent has not been as extensively studied. To broaden our understanding of the Neolithic transition across Europe, we analyzed eight ancient genomes: six samples (four to ∼1- to 4-fold coverage) from a 3,500 year temporal transect (∼8,300–4,800 calibrated years before present) through the Baltic region dating from the Mesolithic to the Late Neolithic and two samples spanning the Mesolithic-Neolithic boundary from the Dnieper Rapids region of Ukraine. We find evidence that some hunter-gatherer ancestry persisted across the Neolithic transition in both regions. However, we also find signals consistent with influxes of non-local people, most likely from northern Eurasia and the Pontic Steppe. During the Late Neolithic, this Steppe-related impact coincides with the proposed emergence of Indo-European languages in the Baltic region [3, 4]. These influences are distinct from the early farmer admixture that transformed the genetic landscape of central Europe, suggesting that changes associated with the Neolithic package in the Baltic were not driven by the same Anatolian-sourced genetic exchange.

RESULTS

Sample Context and Genomic Data

In Europe, the Neolithic transition marked the beginning of a period of innovations that saw communities shift from a mobile lifestyle, dependent on hunting and gathering for survival, to a more sedentary way of life based on food production. This new lifeway, which originated in the Near East ∼11,500 calibrated years before present (cal BP) [5, 6], had arrived in southeast Europe by ∼8,500 cal BP [7], from where it spread quickly across the continental interior of Europe and introduced animal husbandry, cultivated cereals, pottery, and ground stone tools to the region. There is a long-standing debate among archaeologists whether this spread was due to the dispersal of farmers into new lands (i.e., demic diffusion) or horizontal cultural transmission [8]. Genetic evidence suggests that these cultural and technological changes were accompanied by profound genomic transformation, consistent with the migration of people of most likely Anatolian origin [9–12]. In contrast to central Europe, the adoption of agriculture in northern and eastern parts of this continent, in the areas which encompass modern-day Latvia and Ukraine, was slow and relatively recent [13–16]. Although some features of the Neolithic package, such as ceramics, appeared as early as 8,500–7,500 cal BP [17, 18], agriculture was not adopted as a primary subsistence economy until the Late Neolithic/Bronze Age [13–16, 19].

The Neolithic transition in the Baltic and Ukraine thus had a different tempo to that of central Europe, and it is unclear how this may have shaped the genetic composition of these regions. To investigate this, we sampled three Mesolithic and three Neolithic individuals from the archaeological site of Zvejnieki (Latvia), which is one of the richest Stone Age cemeteries in Northern Europe for number of inhumations, as well as duration of use [20, 21] (see the Supplemental Experimental Procedures for site details). We also sampled a Mesolithic and a Neolithic individual from cemeteries found along the Dnieper River in Ukraine (Vasilyevka 3 and Vovnigi 2, respectively). DNA was extracted from the petrous portion of the temporal bone (see the Experimental Procedures), which yielded between 4.30% and 55.99% endogenous DNA for all samples. Samples were shotgun sequenced using Illumina sequencing technology to between 0.22- and 4.37-fold coverage (Figure 1). The authenticity of the data was assessed in silico by examining the data for signatures of post-mortem DNA damage and evaluating the mitochondrial contamination rate in all samples along with the X chromosome contamination rate in males (see the

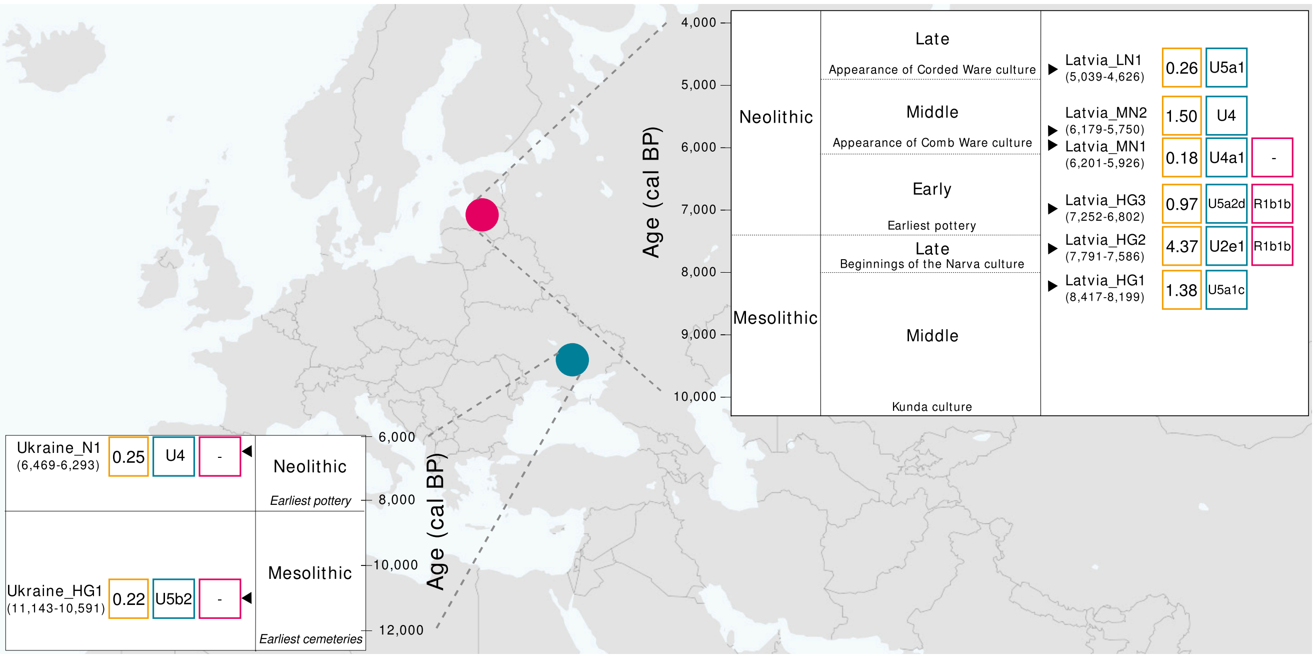

Figure 1. Geographic Location and Chronologies for Latvian and Ukrainian Sites

Radiocarbon dates (in cal BP) are shown under the sample name. Mean genome coverage is shown in yellow squares, mitochondrial haplogroups in blue squares, and Y chromosome haplogroups for male samples (where discernible) in magenta squares. The chronology of the Latvian site of Zvejnieki is adapted from [21]. The Ukrainian chronology is taken from [18, 22, 23].

Supplemental Experimental Procedures). All samples had degradation patterns typical of ancient DNA (Figure S1) and low contamination estimates of ~1% or less (Table 1).

Episodes of Continuity and Change during the Mesolithic and Neolithic in the Baltic

The two earliest samples in our Baltic time series, Latvia_HG1 (8,417–8,199 cal BP), associated with the Kunda culture, and Latvia_HG2 (7,791–7,586 cal BP), associated with the Narva culture, derive from the Late Mesolithic period [17, 21]. A third sample, Latvia_HG3 (7,252–6,802 cal BP), dates to the Late Mesolithic/Early Neolithic period, with the burial showing no major departures from the preceding Mesolithic traditions [21]. Principal component analysis (PCA) with ancient samples projected onto modern Eurasian genetic variation (see the Supplemental Experimental Procedures) shows that these three hunter-gatherer samples group together in a PCA plot (first two components, Figures 2A and S1A). In keeping with their geographical origins, they are in an intermediate position between Western European hunter-gatherer samples (WHG; from Luxembourg, Hungary, Italy, France, and Switzerland) and Eastern European hunter-gatherer samples (EHG; from Russia). They are composed of the same (blue) major component as these other hunter-gatherer groups in an ancestry coefficient decomposition analysis performed using ADMIXTURE [25] (Figure 2B), suggesting a close relationship between these groups. We found that although the Latvian Mesolithic samples share closer affinity to WHG than to EHG, the Latvian Mesolithic samples do not belong entirely to either hunter-gatherer group (tested using *D* statistics [27], which offer a formal test of admixture; Table 2). This suggests that they may be a previously unsampled component of a hunter-gatherer meta-population that stretched across Northern Europe during the early Holocene.

Next we sampled two Middle Neolithic individuals, Latvia_MN1 (6,201–5,926 cal BP), from an isolated grave located among burials from earlier periods, and Latvia_MN2 (6,179–5,750 cal BP), who was interred in a collective burial with five other individuals. During the Middle Neolithic at Zvejnieki, mortuary practices from the preceding periods were partially maintained, but some new features appeared, including collective burials and votive deposits, which are associated with the Comb Ware culture or its influences in the Baltic [21]. Despite having been roughly contemporaneous, these Middle Neolithic samples cluster in different regions of our PCA plot (Figure 2A) and have distinct profiles in ADMIXTURE analysis (Figure 2B). In both analyses, Latvia_MN1 groups with the Mesolithic Latvian samples, suggesting a degree of continuity across the Mesolithic-Neolithic transition in this region and consistent with suggestions that the eastern Baltic was a genetic refugium for hunter-gatherer populations during the Neolithic period [28]. The persistence of hunter-gatherer ancestry in the Baltic until at least the Middle Neolithic also provides a possible source for the resurgence of hunter-gatherer ancestry that is proposed to have occurred in central Europe from 7,000–5,000 cal BP [1]. In contrast, Latvia_MN2 is placed toward EHG in PCA space and has several components in ADMIXTURE analysis that are found in Native Americans, Siberians, and hunter-gatherer samples from the Caucasus. In keeping with these results, we found that there has been a northern Eurasian influence in the Baltic region since the Mesolithic period, as suggested by significantly positive statistics for the test *D*(Mbuti, *X*; Latvia Mesolithic, Latvia_MN2) when *X* was an EHG, modern and ancient Siberian

Table 1. Alignment and Contamination Results for Latvian and Ukrainian Ancient Samples

Sample	Site	Context	Burial	Aligned Reads	Aligned Reads (%)	MT Coverage	MT Contamination (c+md/c−md)	X Contamination (Test 1/Test 2)
Latvia_HG1	Zvejnieki	Mesolithic; Kunda culture	313	54,784,565	49.26	47.83	0.68/0.04	–
Latvia_HG2	Zvejnieki	Mesolithic; Narva culture	93	172,707,718	55.99	114.97	0.94/0.19	0.92 ± 0.08/0.88 ± 0.17
Latvia_HG3	Zvejnieki	Mesolithic/Early Neolithic	121	37,749,963	45.51	40.29	0.77/0.10	0.99 ± 0.26/0.72 ± 0.37
Latvia_MN1	Zvejnieki	Middle Neolithic	124	6,648,453	5.22	8.14	0.97/0.50	–
Latvia_MN2	Zvejnieki	Middle Neolithic; Comb Ware culture	221	59,800,396	37.51	48.54	0.69/0.10	–
Latvia_LN1	Zvejnieki	Late Neolithic; Corded Ware culture	137	9,222,060	7.48	9.58	1.09/0.00	–
Ukraine_HG1	Vasilyevka	Mesolithic	37	9,528,908	4.30	5.49	0.29/0.29	–
Ukraine_N1	Vovnigi	Neolithic; Dnieper-Donets culture	2	10,741,415	12.04	6.06	1.06 0.28	–

MT, mitochondria; c+md, percentage contamination including sites with potentially damaged bases; c−md, percentage contamination excluding sites with potentially damaged bases. For X chromosome contamination estimation, two tests were performed as described by Rasmussen et al. [24]. Test 1 used all high-quality reads provided per sample, whereas only a single read was sampled per site for test 2, thereby removing the assumption of independent error rates. The associated p values calculated using Fisher's exact test [24] were ≤ 0.05 for all tests. See also Table S1 for imputed genotypes probabilities for selected loci.

(including the Upper Palaeolithic Mal'ta genome [29]), or Native American (Table 2). This influence is supported archaeologically by the appearance of copper rings and amber jewelry in Middle Neolithic collective burials that bear similarities to artifacts found in Estonia, Finland, and northwestern Russia [21, 30].

The latest Neolithic sample in our Baltic time series, Latvia_LN1 (5,039–4,626 cal BP), which was found in a crouched burial of the type associated with the Late Neolithic Corded Ware culture [21], falls near other Late Neolithic and Bronze Age European and Steppe samples in PCA analysis (Figure 2A). In ADMIXTURE analysis, it is composed of the blue component (Figure 2B), which is predominant in all of the older Latvian samples, but also a green component, which is maximized in hunter-gatherer samples from the Caucasus. A Caucasus-related influence in this sample is also suggested by positive results (although without formal significance, $Z > 2$) for tests of the form *D*(Mbuti, Caucasus hunter-gatherer; Latvian Mesolithic, Latvia_LN1). Ancestry related to hunter-gatherers from the Caucasus has previously been postulated to have arrived in Europe through herders from the Pontic Steppe [1, 31], and these migrations could potentially be the source of this ancestry in our sample. Interestingly, this individual lived around the time of later date estimates (~4,500–7,000 cal BP) proposed for the split of Proto-Balto-Slavic from other Indo-European languages [3, 4]. There are two major theories to explain the distribution of Indo-European languages that constitute the most widely spoken language family in the world: (1) they have an Anatolian origin and were spread by Neolithic agriculturalists [32, 33] and (2) they developed in the Pontic Steppe and proliferated through Late Neolithic/Bronze Age migrations [1, 3, 34]. The presence of a Steppe-related component in Latvia_LN1 in the absence of an Anatolian farmer-related genetic input supports a Steppe rather than an Anatolian origin for the Balto-Slavic branch of the Indo-European language family.

It is striking that we did not find evidence for early European or Anatolian farmer admixture in any of our Latvian Neolithic samples using both *D* statistics (Table 2) and ADMIXTURE (Figure 2A). This lack of admixture is also supported by the mitochondrial haplogroup of the Latvian Neolithic samples (all belong to U; Figure 1), which is prevalent in European hunter-gatherers [1, 35], including our Latvian Mesolithic samples, but not in early farmers. It is interesting that among the grave goods found in the burial of Latvia_LN1 was a chisel made from the bone of a domesticated goat or sheep [17, 21]. The presence of this tool made from a domesticate as well as dietary isotope data (δ^{15}N and δ^{13}C), which show greater reliance on terrestrial resources than in previous periods [17], is consistent with either the adoption of farming without early European farmer-related genetic admixture or the existence of trade networks with farming communities that were largely independent of genomic exchange. Although we find no genetic input from Anatolian or early European farmers in our time series, ADMIXTURE analysis of an Estonian Corded Ware sample [26] (Figure 2B) has suggested that this farmer genetic influence, which is present in contemporary Northern European populations (Figure S2), had arrived in the Baltic by at least the Bronze Age.

The Neolithic Transition in Ukraine

The Ukrainian Mesolithic and Neolithic male samples (Ukraine_HG1 [11,143–10,591 cal BP] and Ukraine_N1 [6,469–6,293 cal BP], respectively) cluster tightly together between WHG and EHG samples in PCA analysis (Figure 2A). They form a clade with respect to other modern and ancient samples when tested using genome-wide *D* statistics (*D*(Mbuti, *X*; Ukraine_HG1, Ukraine_N1); Table S1), and their mitochondria belong to the U haplogroup, which has been found in ~80% of European hunter-gatherer samples [1, 35]. These results suggest a degree of continuity across 4,000 years from the Mesolithic to the Neolithic period in the Dnieper Rapids. In ADMIXTURE analysis (Figure 2B), both Ukrainian samples are composed almost entirely of the European hunter-gatherer (blue) component, with a smaller green component that is also found in EHG. This green component is slightly larger in the Neolithic sample than in the Mesolithic sample, which is

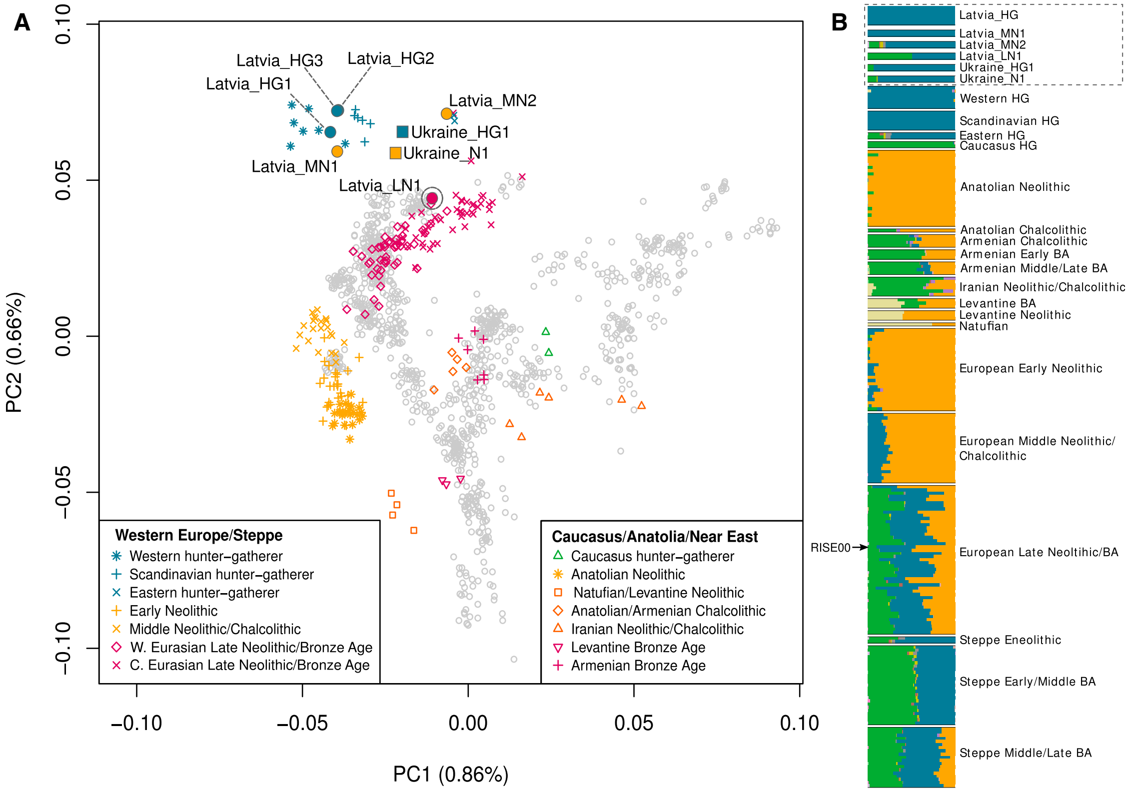

Figure 2. PCA and ADMIXTURE Analysis for Ancient Latvian and Ukrainian Samples

(A) Ancient data presented in this study as well as published ancient data (see Data S1 for sample details) were projected onto the first two principal components defined by selected modern Eurasians from the Human Origins dataset (see the Supplemental Experimental Procedures). Our Latvian Mesolithic samples cluster tightly together between western and eastern hunter-gatherers in PCA space, whereas the Latvian Neolithic samples are more variable in their position, suggesting impacts from exogenous populations. The Ukrainian Mesolithic and Neolithic samples fall close together between western and eastern hunter-gatherers, suggesting a degree of continuity across the Mesolithic-Neolithic boundary in this region.

(B) ADMIXTURE ancestry components ($K = 17$) [25] for ancient samples showing that the Latvian Neolithic samples do not have the yellow component that dominates in Anatolian and early European farmers. The Latvian and Ukrainian samples presented in this study are displayed in a gray box and at twice the height of the other ancient samples for ease of visualization. The arrow shows an Estonian Bronze Age sample (RISE00) [26] that has a yellow component, suggesting that an early European farmer genetic influence had arrived in the Baltic by the Bronze Age.

HG, hunter-gatherer; BA, Bronze Age; W, western; C, Central. See also Figures S1–S4.

in keeping with *D* statistics that suggest increased affinity with ancient northern Eurasians from the Mesolithic to the Neolithic in Ukraine (Table S1). It is intriguing that we find an increased affinity to northern Eurasian samples in both Ukraine and Latvia during the Neolithic period. This could be the result of increased connectivity in Europe at this time. More extensive sampling will reveal whether this is a feature of the Neolithic across Northern and Eastern Europe.

Relationship of Ancient Samples to Modern Populations

The ancient Latvian and Ukrainian samples fall close to modern Northern and Eastern European populations in PCA analysis (Figures 2A and S1A), suggesting a degree of continuity in both regions since the Mesolithic period. Outgroup f_3 statistics, which measure shared genetic drift between populations, further support this as they show that these ancient samples share most affinity with modern populations from Northern and Eastern Europe (Figure S3). Further, the Y chromosomes of two of our Latvian Mesolithic samples were assigned to haplogroup R1b (the maximum-likelihood sub-haplogroup is R1b1b), which is the most common haplogroup found in modern Western Europeans [36]. This haplogroup has been found at low frequencies before the Late Neolithic in Western Europe [1, 35] but at higher frequencies in Russia and is suggested to have spread into Europe from the East after 5,000 cal BP [1]. The presence of this haplogroup in Mesolithic Latvia points to a more westward ancestral range. We found that the three Mesolithic Latvian samples are predicted to have had the derived variant (rs12913832) of the *HERC2* gene associated with blue eye color (Figure S3B and Table S2). Blue eye color is found at high frequencies in Northern Europe today, and these results suggest that this phenotype was already present in the Baltic by the Mesolithic period. We also found tentative evidence for progressive skin depigmentation in Latvia based on mutations in the *SLC24A5* and *SLC45A2* genes (rs1426654 and rs16891982, respectively; Figure S3B and Table S2).

DISCUSSION

The Neolithic transitions in the Baltic and Dnieper Rapids region of Ukraine show very different archaeological and genetic dynamics to those observed in Central and Western Europe. Although in central Europe pottery and agriculture arrive as a package, in the Baltic and Dnieper Rapids the onset of the Neolithic is characterized by the appearance of ceramics, with a definitive shift to an agro-pastoralist economy only occurring during the Late Neolithic/Bronze Age [13–16, 19]. Although the prolonged and piecemeal uptake of Neolithic characteristics in these regions makes it challenging to attribute a definitive shift in ideology or lifestyle, it does, along with evidence for continuities in material culture and settlement patterns, suggest that Neolithic features were predominantly adopted by indigenous hunter-gatherers in this region [13–16, 37]. We find genetic evidence in support of this in the affinity of the Latvian and Ukrainian Neolithic samples, Latvian_MN1 and Ukrainian_N1, to earlier

Table 2. Key *D* Statistics of the Form *D*(*A*,*B*; *X*,*Y*) for Latvian Samples

A	B	X	Y	*D*	Z Score	Loci
Latvian Mesolithic Samples[a]						
Mbuti	**Latvia_HG**	**EHG**	**WHG**	0.0787	13.064	103,420
Mbuti	**EHG**	**Latvia_HG**	**WHG**	−0.0281	−4.797	103,420
Mbuti	**WHG**	**Latvia_HG**	**EHG**	−0.1065	−17.418	103,420
Mbuti	***Latvia_HG***	***Karelia (EHG)***	***Bichon (WHG)***	*0.0801*	*11.725*	*3,188,010*
Mbuti	***Latvia_HG***	***Karelia (EHG)***	***Loschbour (WHG)***	*0.0928*	*13.521*	*3,204,237*
Mbuti	***Karelia (EHG)***	***Latvia_HG***	***Bichon (WHG)***	*−0.0432*	*−6.494*	*3,188,010*
Mbuti	***Karelia (EHG)***	***Latvia_HG***	***Loschbour (WHG)***	*−0.0354*	*−5.367*	*3,204,237*
Mbuti	***Bichon (WHG)***	***Karelia (EHG)***	***Latvia_HG***	*0.1228*	*17.680*	*3,188,010*
Mbuti	***Loschbour (WHG)***	***Karelia (EHG)***	***Latvia_HG***	*0.1277*	*18.643*	*3,204,237*
Latvia_MN1[b]						
Mbuti_AF	**Iran_LN**	**Latvia_HG**	**Latvia_MN1**	0.0427	1.459	7,261
Mbuti_AF	**Anatolia_ChL**	**Latvia_HG**	**Latvia_MN1**	0.0249	0.907	8,289
Mbuti_AF	**Kennewick**	**Latvia_HG**	**Latvia_MN1**	0.0181	0.685	9,026
Latvia_MN2[c]						
Mbuti	Zapotec	**Latvia_HG**	**Latvia_MN2**	0.0295	4.171	74,461
Mbuti	Guarani	**Latvia_HG**	**Latvia_MN2**	0.0303	4.027	74,461
Mbuti	Aymara	**Latvia_HG**	**Latvia_MN2**	0.0282	3.926	74,461
Mbuti	**EHG**	**Latvia_HG**	**Latvia_MN2**	0.0346	3.734	70,000
Mbuti	**AfontovaGora3**	**Latvia_HG**	**Latvia_MN2**	0.0658	3.519	19,541
Mbuti	**MA1**	**Latvia_HG**	**Latvia_MN2**	0.0381	3.441	53,389
Mbuti	***Karelia (EHG)***	***Latvia_HG***	***Latvia_MN2***	*0.0386*	*5.742*	*2,165,498*
Mbuti	***MA1***	***Latvia_HG***	***Latvia_MN2***	*0.0553*	*7.355*	*1,799,638*
Mbuti	*Karitiana*	***Latvia_HG***	***Latvia_MN2***	*0.0366*	*5.307*	*2,693,045*
Latvia_LN1[d]						
Mbuti	**CHG**	**Latvia_HG**	**Latvia_LN1**	0.0312	2.081	20,994
Mbuti	**Iran_N**	**Latvia_HG**	**Latvia_LN1**	0.0275	1.483	16,000
Mbuti	**Iran_ChL**	**Latvia_HG**	**Latvia_LN1**	0.0203	1.457	19,027
Mbuti	**Iran_LN**	**Latvia_HG**	**Latvia_LN1**	0.0319	1.320	9,481
Mbuti	Iranian_Jew_WA	**Latvia_HG**	**Latvia_LN1**	0.0106	1.091	20,998
Mbuti	***Kotias (CHG)***	***Latvia_HG***	***Latvia_LN1***	*0.0182*	*2.138*	*715,061*
Latvian Neolithic Samples[e]						
Mbuti	**Anatolia_N**	**Latvia_HG**	**Latvia_MN1**	−0.0013	−0.108	16,255
Mbuti	**Europe_EN**	**Latvia_HG**	**Latvia_MN1**	−0.0055	−0.476	16,272
Mbuti	**Anatolia_N**	**Latvia_HG**	**Latvia_MN2**	−0.0246	−3.607	74,355
Mbuti	**Europe_EN**	**Latvia_HG**	**Latvia_MN2**	−0.0335	−5.055	74,460
Mbuti	**Anatolia_N**	**Latvia_HG**	**Latvia_LN1**	−0.0221	−2.136	20,969
Mbuti	**Europe_EN**	**Latvia_HG**	**Latvia_LN1**	−0.0308	−2.952	20,998
Mbuti	***Stuttgart***	***Latvia_HG***	***Latvia_MN1***	*0.0168*	*1.804*	*581,303*
Mbuti	***NE1***	***Latvia_HG***	***Latvia_MN1***	*0.0161*	*1.704*	*581,758*
Mbuti	***Stuttgart***	***Latvia_HG***	***Latvia_MN2***	*−0.0300*	*−4.527*	*2,722,280*
Mbuti	***NE1***	***Latvia_HG***	***Latvia_MN2***	*−0.0272*	*−3.871*	*2,724,548*

(*Continued on next page*)

Table 2. *Continued*

A	B	X	Y	D	Z Score	Loci
Mbuti	***Stuttgart***	***Latvia_HG***	***Latvia_LN1***	*−0.0246*	*−2.955*	*715,090*
Mbuti	***NE1***	***Latvia_HG***	***Latvia_LN1***	*−0.0215*	*−2.373*	*715618*

Latvia_HG, Latvian hunter-gatherers; WHG, western hunter-gatherers; EHG, eastern hunter-gatherers; Iran_LN, Iranian Late Neolithic; Anatolia_ChL, Anatolian Chalcolithic; CHG, Caucasus hunter-gatherers; Iran_N, Iranian Neolithic; Iran_ChL, Iranian Chalcolithic; Anatolian_N, Anatolian Neolithic; Europe_EN, European Early Neolithic. Tests performed using the whole genome panel are italicized; otherwise, tests were performed using the Human Origin transversion SNP panel. Ancient samples are shown in bold. Samples include in each ancient group can be found in Data S1. See Table S1 for key *D* statistics for Ukrainian samples.

[a] The Latvian Mesolithic samples share more affinity to WHG than to EHG, but they do not belong entirely to either group.

[b] There is no evidence for admixture in our Latvian Neolithic sample, Latvia_MN1 (the three largest positive statistics are shown).

[c] There is an eastern influence in the Latvian Middle Neolithic sample, Latvia_MN2, as compared to the Latvian Mesolithic samples (the three most significantly positive results with ancient and modern populations/individuals from the Human Origins SNP panel dataset are shown).

[d] Largest positive results for the test *D*(Mbuti, *X*; Latvia_HG, Latvia_LN1). ADMIXTURE results suggest that there may have been a CHG-related influence in Latvia during the Late Neolithic period; however, although *D* statistics to test this are positive, they do not reach significance.

[e] We do not find evidence for early European/Anatolian farmer admixture in our Latvian Neolithic samples.

Mesolithic samples from the same respective regions. However, we also find indications of genetic impact from exogenous populations during the Neolithic, most likely from northern Eurasia and the Pontic Steppe. These influences are distinct from the Anatolian-farmer-related gene flow found in central Europe during this period. It is interesting to note that even in outlying areas of Europe, such as Sweden and Ireland [38, 39], an Anatolian-farmer-related genetic signature is present by the Middle to Late Neolithic period (~5,300–4,700 cal BP). We conclude that the gradual appearance of features associated with the Neolithic package in the Baltic and Dnieper Rapids was not tied to the same major genetic changes as in other regions of Europe. The emergence of Neolithic features in the absence of immigration by Anatolian farmers highlights the roles of horizontal cultural transmission and potentially independent innovation during the Neolithic transition.

EXPERIMENTAL PROCEDURES

DNA was extracted from a petrous bone of our samples in dedicated ancient DNA facilities (see the Supplemental Experimental Procedures). Libraries were prepared and sequenced using 50–100 bp Illumina single-end sequencing, and reads were aligned to the GRCh37 build of the human genome with the mitochondrial sequence replaced by the revised Cambridge reference sequence. The authenticity of the data was assessed by looking for short average sequence length and patterns of molecular damage and estimating the mitochondrial and X chromosome contamination rates (see the Supplemental Experimental Procedures). Sex was determined by examining the ratio of Y chromosome reads to reads aligning to both sex chromosomes [40]. Pseudo-diploid genotypes were called in our sample at positions that overlapped autosomal genotypes in the Human Origins dataset (110,507 positions) and merged with publicly available ancient genotypes (see the Supplemental Experimental Procedures). The relationship between our ancient samples and other modern and ancient populations was assessed using PCA, ADMIXTURE, *D* statistics, and f_3 statistics (see the Supplemental Experimental Procedures). Mitochondrial and Y chromosome haplogroups were determined using HAPLOFIND and Yfitter, respectively. Genotypes associated with particular phenotypes were examined using observed and imputed genotypes as in [2] (see the Supplemental Experimental Procedures, Figure S3, and Table S2).

ACCESSION NUMBERS

The accession number for the sequence data reported in this paper is European Nucleotide Archive (ENA): PRJEB18067.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, two tables, and one data file and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.12.060.

AUTHOR CONTRIBUTIONS

D.G.B., R.P., and A.M. supervised the study. E.R.J., D.G.B., and A.M. analyzed genetic data. R.P., G.Z., V.M., and P.R.N. provided samples and/or input about archaeological context. E.L. helped interpret isotope analyses. E.R.J., A.M., P.R.N., and D.G.B. wrote the manuscript with input from all co-authors.

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Supplemental Information

The Neolithic Transition in the Baltic Was Not Driven by Admixture with Early European Farmers

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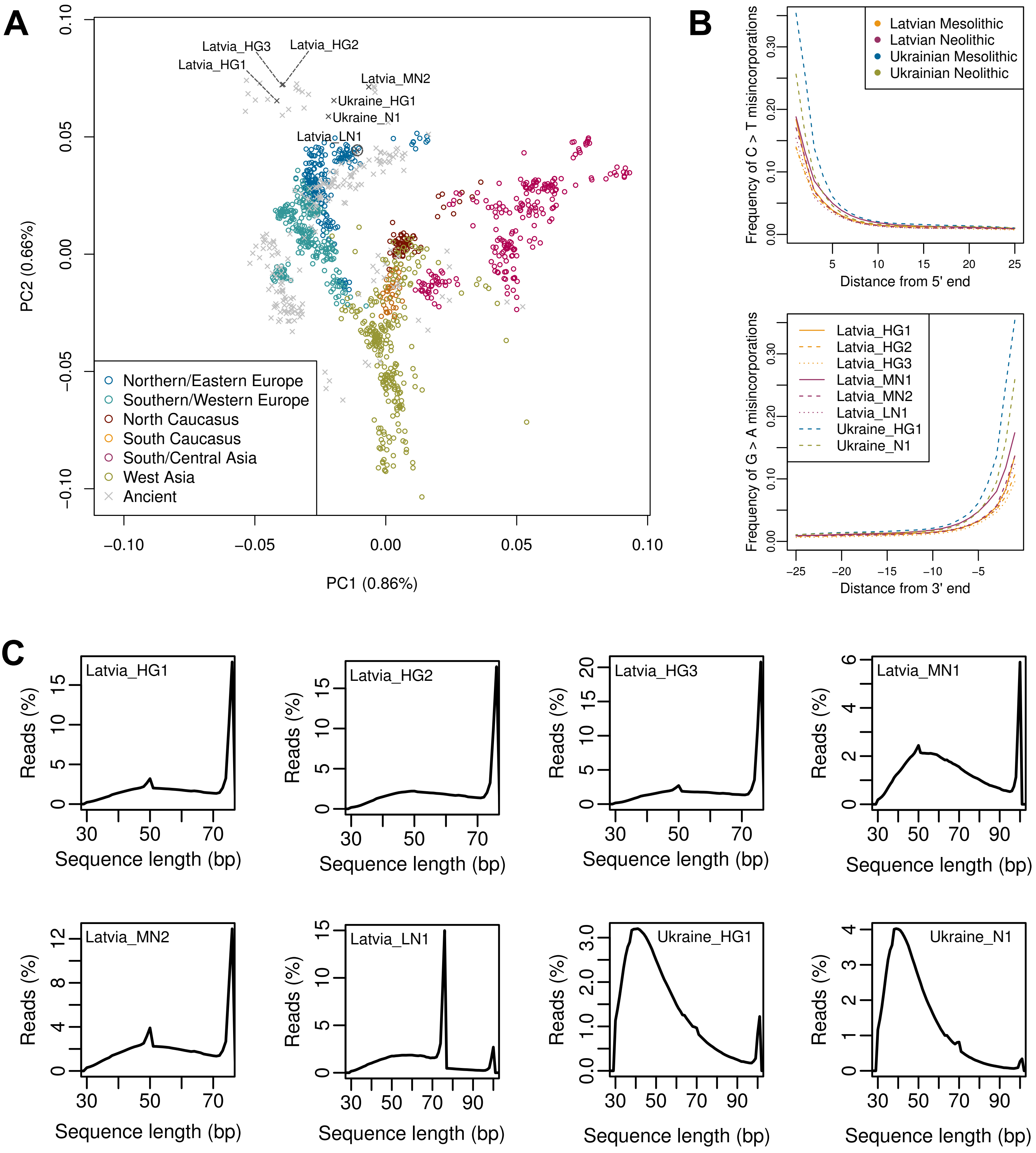
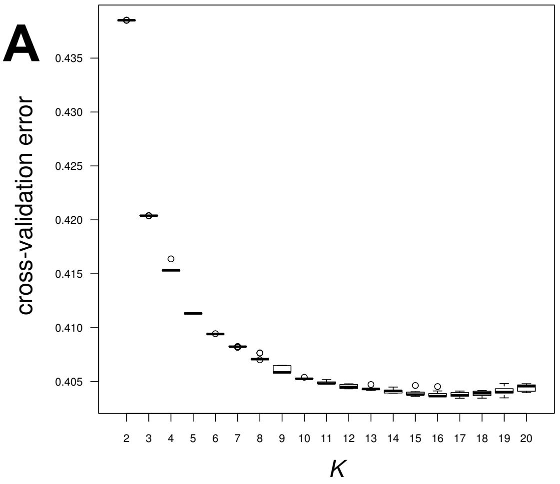
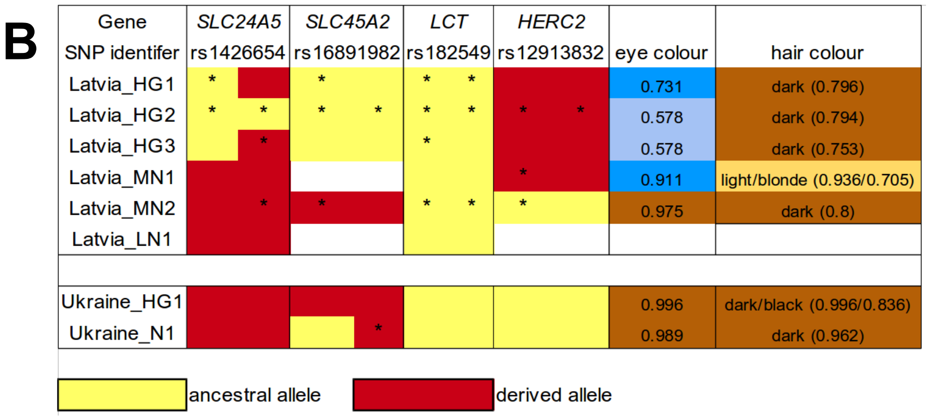


Figure S1. PCA and patterns of molecular damage. Related to Figure 2. A. PCA with modern individuals shown in colour and ancient individuals shown in grey. Datapoints are the same as in Figure 2A. B. Damage patterns for ancient samples. Plots show mismatch frequency relative to the reference genome as a function of read position for Latvian and Ukrainian samples. C. Sequence length distribution plots for ancient samples. Some samples have blips in their distributions as they were sequenced with more than one cycling format.

Figure S2. ADMIXTURE analysis showing modern individuals only. Related to Figure 2. 2-20 clusters (K) are shown.

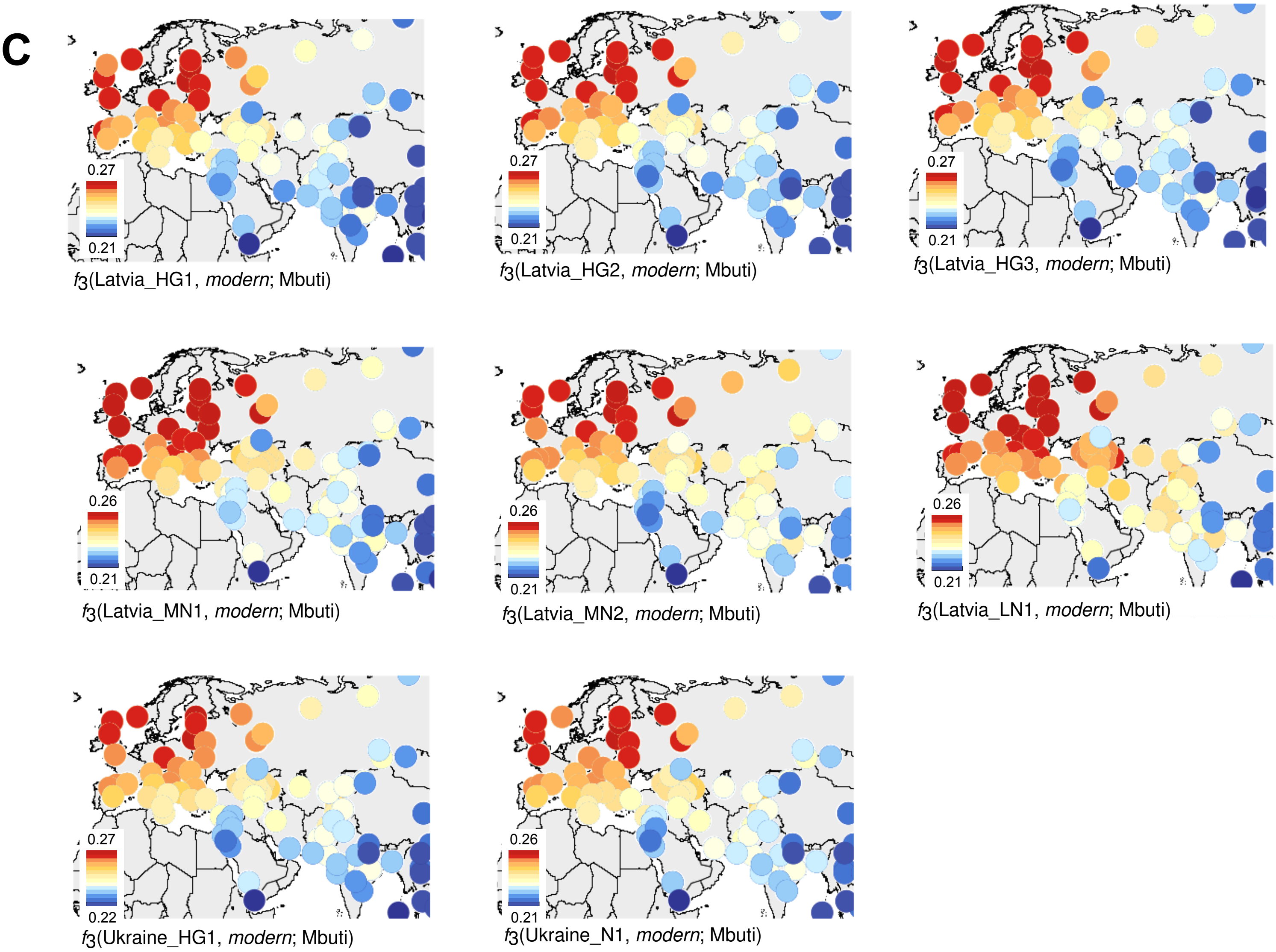




B

Gene	*SLC24A5*	*SLC45A2*	*LCT*	*HERC2*		
SNP identifer	rs1426654	rs16891982	rs182549	rs12913832	eye colour	hair colour
Latvia_HG1	🟨* 🟥	🟨* 🟨	🟨* 🟨*	🟥 🟥	0.731	dark (0.796)
Latvia_HG2	🟨* 🟨*	🟨* 🟨*	🟨* 🟨*	🟥* 🟥*	0.578	dark (0.794)
Latvia_HG3	🟨 🟥*	🟨 🟨	🟨* 🟨	🟥 🟥	0.578	dark (0.753)
Latvia_MN1	🟥 🟥		🟨 🟨	🟥* 🟥	0.911	light/blonde (0.936/0.705)
Latvia_MN2	🟥 🟥*	🟥* 🟥	🟨* 🟨*	🟨* 🟨	0.975	dark (0.8)
Latvia_LN1	🟥 🟥		🟨 🟨			
Ukraine_HG1	🟥 🟥	🟥 🟥	🟨 🟨	🟨 🟨	0.996	dark/black (0.996/0.836)
Ukraine_N1	🟥 🟥	🟨 🟥*	🟨 🟨	🟨 🟨	0.989	dark (0.962)

🟨 ancestral allele 🟥 derived allele



f_3(Latvia_HG1, *modern*; Mbuti) f_3(Latvia_HG2, *modern*; Mbuti) f_3(Latvia_HG3, *modern*; Mbuti)

f_3(Latvia_MN1, *modern*; Mbuti) f_3(Latvia_MN2, *modern*; Mbuti) f_3(Latvia_LN1, *modern*; Mbuti)

f_3(Ukraine_HG1, *modern*; Mbuti) f_3(Ukraine_N1, *modern*; Mbuti)

Figure S3. Cross-validation error for ADMIXTURE analysis, imputed genotypes and outgroup f_3-statistics. Related to Figure 2. A. ADMIXTURE analysis cross validation error as a function of the number of clusters (*K*). 10 replicates were performed for each value of *K*. The minimal error was found at *K*=17, but the error already started plateauing from roughly *K*=10, implying little improvement from this value onwards. B. Selected imputed genotypes (probability ≥ 0.7) for ancient Latvian and Ukrainian samples along with predicted hair and eye colour. The derived alleles in the *SLC24A5* and *SLC45A2* genes are associated with skin de-pigmenation [S1,S2] and *HERC2* with light iris colour [S3,S4]. The derived allele of the *LCT* gene is associated with the ability to digest lactose into adulthood [S5]. Hair and eye colour were predicted using the Hirisplex model [S6]. Stars show imputed genotypes which are supported by observed alleles. Two stars show that the genotype is supported by at least 2 reads, one star shows that the genotype is supported by one read. C. Outgroup f_3-statistics for Latvian and Ukrainian ancient samples. Statistics show that all samples share the most affinity to modern populations from Northern and Eastern Europe.

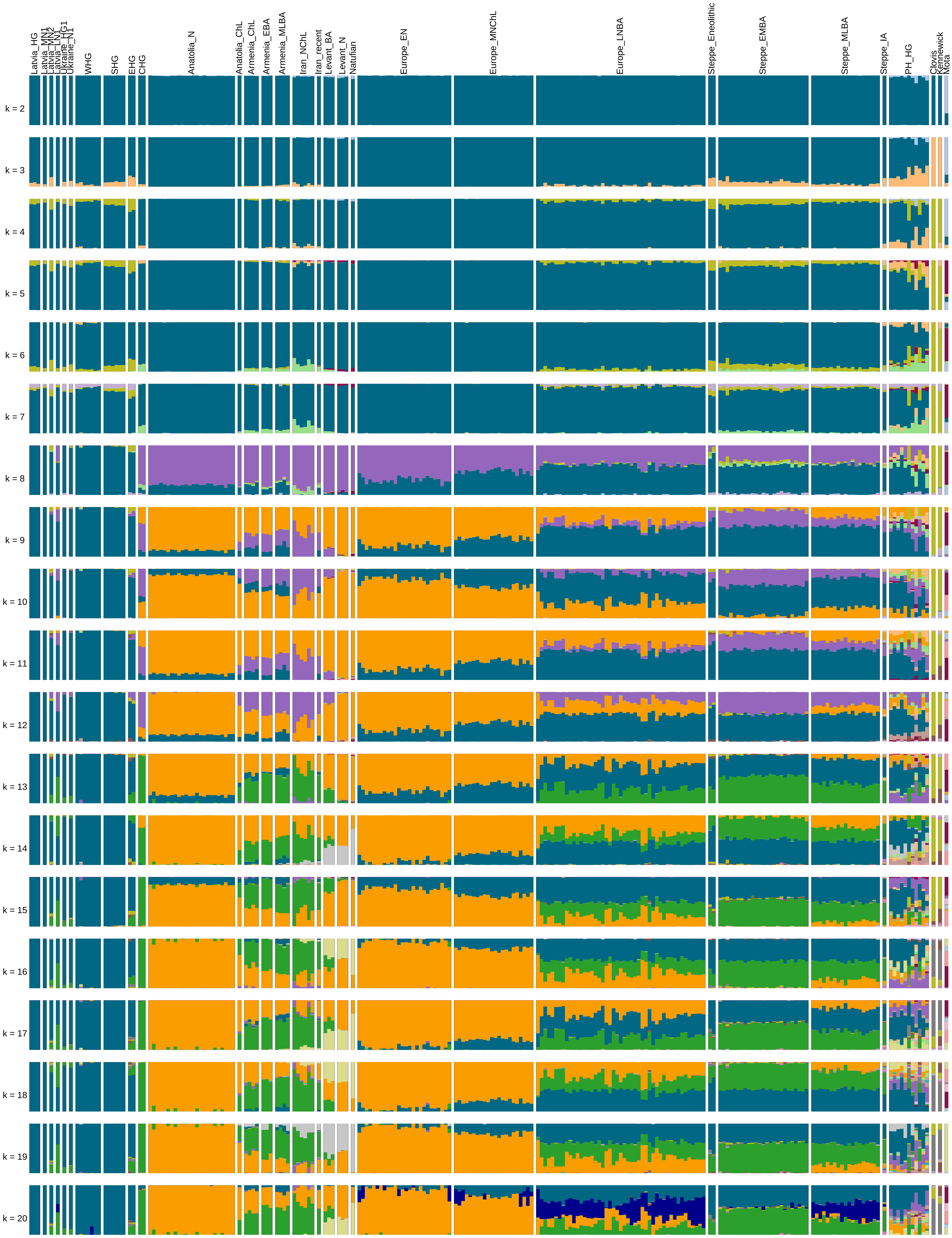


Figure S4. ADMIXTURE analysis showing ancient individuals only. Related to Figure 2. 2-20 clusters (K) are shown.

Table S1. Imputed genotypes probabilities for the SNP panel used in the Hirisplex prediction system.

				Latvia_HG1		**Latvia_HG2**		**Latvia_HG3**		**Latvia_MN1**		**Latvia_MN2**		**Latvia_LN1**		**Ukraine_HG1**		**Ukraine_N1**	
Gene	**SNP identifier**	**allele 0**	**allele 1**	**geno**	**P**	**geno**	**P**	**geno**	**P**	**geno**	**P**	**geno**	**P**	**geno**	**P**	**geno**	**P**	**geno**	**P**
TYR	rs1042602	C	A	0/0	0.932	0/0	1	0/0	1	0/0	0.821	0/0	0.997	0/0	0.989	0/0	0.992	0/0	1
MC1R	rs1110400	T	C	0/0	1	0/0	1	0/0	1	0/0	1	0/0	1	0/0	1	0/0	0.999	0/0	1
MC1R	rs11547464	G	A	0/0	0.999	0/0	0.999	0/0	0.98	0/0	0.982	0/0	1	0/0	0.983	0/0	0.994	0/0	0.985
IRF4	rs12203592	C	T	0/1	0.529	0/1	0.616	0/1	0.531	0/0	0.797	0/0	0.993	0/0	0.975	0/0	0.853	0/0	0.646
KITLG	rs12821256	T	C	0/0	0.997	0/0	1	0/0	0.999	0/0	0.984	0/0	1	0/0	0.969	0/0	0.992	0/0	0.934
SLC24A4	rs12896399	G	T	0/0	1	0/1	0.984	0/1	1	0/1	0.995	0/0	1	0/0	0.664	0/1	0.859	0/0	0.778
HERC2	rs12913832	A	G	1/1	0.848	1/1	0.995	1/1	0.773	1/1	0.761	0/0	1	0/1	0.523	0/0	0.742	0/0	0.969
TYR	rs1393350	G	A	0/0	1	0/0	1	0/0	1	0/0	0.997	0/0	1	0/0	1	0/0	1	0/0	0.988
SLC45A2	rs16891982	C	G	0/0	0.999	0/0	0.999	0/0	0.883	0/1	0.643	1/1	0.997	0/0	0.528	0/0	0.964	0/1	1
OCA2	rs1800407	C	T	0/0	1	0/0	1	0/0	1	0/0	0.998	0/0	1	0/0	0.993	0/1	0.528	0/0	0.998
MC1R	rs1805005	G	T	0/0	0.963	0/0	0.976	0/0	0.814	0/0	0.794	0/0	0.997	0/0	0.875	0/0	0.869	0/0	0.676
MC1R	rs1805006	C	A	0/0	1	0/0	1	0/0	1	0/0	0.998	0/0	1	0/0	1	0/0	0.972	0/0	1
MC1R	rs1805007	C	T	0/0	0.993	0/0	0.999	0/0	0.972	0/0	0.974	0/0	1	0/1	0.53	0/0	0.966	0/0	0.979
MC1R	rs1805008	C	T	0/0	1	0/0	1	0/0	1	0/0	0.99	0/0	0.997	0/0	0.999	0/0	0.985	0/0	0.997
MCIR	rs1805009	G	C	0/0	1	0/0	1	0/0	1	0/0	0.986	0/0	0.995	0/0	0.998	0/0	0.987	0/0	0.987
MC1R	rs2228479	G	A	0/0	1	0/0	1	0/0	1	0/0	0.974	0/0	0.999	0/0	1	0/0	0.996	0/0	0.991
PIGU/ASIP	rs2378249	G	A	1/1	1	1/1	1	1/1	1	1/1	0.999	1/1	1	1/1	0.997	1/1	0.999	0/1	0.995
SLC24A4	rs2402130	G	A	1/1	1	1/1	0.999	1/1	1	1/1	0.795	1/1	1	0/1	0.98	0/1	0.871	1/1	0.981
SLC45A2	rs28777	C	A	0/0	0.997	0/0	0.951	0/0	0.842	1/1	0.538	1/1	1	0/1	0.626	0/0	0.957	0/1	0.998
EXOC2	rs4959270	C	A	0/1	0.999	0/1	1	1/1	0.998	0/0	0.563	0/0	1	0/1	0.99	0/1	0.702	1/1	0.951
TYRP1	rs683	C	A	0/1	1	0/1	1	1/1	1	0/1	0.999	1/1	1	1/1	0.989	0/0	1	0/1	0.997
MC1R	rs86insA	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MC1R	rs885479	G	A	0/0	1	0/0	1	0/0	1	0/0	0.962	0/1	0.703	0/0	0.969	0/0	0.929	0/0	0.891
MC1R	Y152OCH	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

geno, genotype; P, probability

Sample details are shown in Table 1.

Table S2. Key *D*-statistics of the form *D*(*A*,*B*; *X*,*Y*) for Ukrainian samples.

A	**B**	**X**	**Y**	**D**	**Z-score**	**Loci**
The Ukrainian samples form a clade to the exclusion of other tested samples.						
Mbuti	***Loschbour (WHG)***	***Ukraine_HG1***	***Ukraine_N1***	*-0.0453*	*-2.298*	*133784*
Mbuti	***Latvia_LN1***	***Ukraine_HG1***	***Ukraine_N1***	*-0.0703*	*-1.526*	*25338*
Mbuti	***BR2***	***Ukraine_HG1***	***Ukraine_N1***	*-0.0264*	*-1.357*	*133824*
Mbuti	***Kotias (CHG)***	***Ukraine_HG1***	***Ukraine_N1***	*-0.0258*	*-1.328*	*133744*
Mbuti	***Bichon (WHG)***	***Ukraine_HG1***	***Ukraine_N1***	*-0.0238*	*-1.144*	*132164*
Mbuti	*Sardinian*	***Ukraine_HG1***	***Ukraine_N1***	*-0.0205*	*-0.997*	*132316*
Mbuti	*Dinka*	***Ukraine_HG1***	***Ukraine_N1***	*-0.0206*	*-0.926*	*133142*
Mbuti	*Han*	***Ukraine_HG1***	***Ukraine_N1***	*-0.0173*	*-0.807*	*132983*
Mbuti	***Latvia_HG***	***Ukraine_HG1***	***Ukraine_N1***	*-0.0125*	*-0.717*	*133260*
Mbuti	***Stuttgart***	***Ukraine_HG1***	***Ukraine_N1***	*-0.0140*	*-0.701*	*133719*
Mbuti	*Karitiana*	***Ukraine_HG1***	***Ukraine_N1***	*-0.0122*	*-0.567*	*132697*
Mbuti	*Mandenka*	***Ukraine_HG1***	***Ukraine_N1***	*-0.0113*	*-0.534*	*132062*
Mbuti	*French*	***Ukraine_HG1***	***Ukraine_N1***	*-0.0107*	*-0.513*	*133120*
Mbuti	***Latvia_MN1***	***Ukraine_HG1***	***Ukraine_N1***	*-0.0160*	*-0.370*	*21119*
Mbuti	*Yoruba*	***Ukraine_HG1***	***Ukraine_N1***	*0.0005*	*0.022*	*133213*
Mbuti	*Dai*	***Ukraine_HG1***	***Ukraine_N1***	*0.0028*	*0.121*	*132618*
Mbuti	***NE1***	***Ukraine_HG1***	***Ukraine_N1***	*0.0033*	*0.164*	*133836*
Mbuti	***Karelia (EHG)***	***Ukraine_HG1***	***Ukraine_N1***	*0.0062*	*0.290*	*103705*
Mbuti	***Latvia_MN2***	***Ukraine_HG1***	***Ukraine_N1***	*0.0085*	*0.355*	*98909*
Mbuti	*San*	***Ukraine_HG1***	***Ukraine_N1***	*0.0112*	*0.506*	*133136*
Mbuti	***MA1***	***Ukraine_HG1***	***Ukraine_N1***	*0.0156*	*0.636*	*84801*
Mbuti	*Papuan*	***Ukraine_HG1***	***Ukraine_N1***	*0.0265*	*1.257*	*132572*
There may have been increased connectivity with northern Eurasia from the Mesolithic to the Neolithic in Ukraine.						
Mbuti	**AfontovaGora3**	**WHG**	**Ukraine_HG1**	0.0846	2.411	4087
Mbuti	**AfontovaGora3**	**WHG**	**Ukraine_N1**	0.1462	4.461	4310
Mbuti	**MA1**	**WHG**	**Ukraine_HG1**	0.0032	0.158	11652
Mbuti	**MA1**	**WHG**	**Ukraine_N1**	0.0708	3.369	11892
Mbuti	**EHG**	**WHG**	**Ukraine_HG1**	0.0237	1.417	15359
Mbuti	**EHG**	**WHG**	**Ukraine_N1**	0.0421	2.546	15634
Mbuti	**CHG**	**WHG**	**Ukraine_HG1**	0.0143	0.915	16477
Mbuti	**CHG**	**WHG**	**Ukraine_N1**	0.0140	0.886	16724
Mbuti	***MA1***	***Loschbour (WHG)***	***Ukraine_HG1***	*0.0114*	*0.936*	*447952*
Mbuti	***MA1***	***Loschbour (WHG)***	***Ukraine_N1***	*0.0510*	*4.179*	*439711*
Mbuti	***MA1***	***Bichon (WHG)***	***Ukraine_HG1***	*0.0062*	*0.520*	*444959*
Mbuti	***MA1***	***Bichon (WHG)***	***Ukraine_N1***	*0.0509*	*4.102*	*437042*
Mbuti	***Karelia (EHG)***	***Loschbour (WHG)***	***Ukraine_HG1***	*0.0269*	*2.451*	*546828*
Mbuti	***Karelia (EHG)***	***Loschbour (WHG)***	***Ukraine_N1***	*0.0491*	*4.426*	*531401*
Mbuti	***Karelia (EHG)***	***Bichon (WHG)***	***Ukraine_HG1***	*0.0348*	*3.069*	*543214*
Mbuti	***Karelia (EHG)***	***Bichon (WHG)***	***Ukraine_N1***	*0.0560*	*4.998*	*528197*
Mbuti	***Kotias (CHG)***	***Loschbour (WHG)***	***Ukraine_HG1***	*0.0016*	*0.155*	*695299*
Mbuti	***Kotias (CHG)***	***Loschbour (WHG)***	***Ukraine_N1***	*0.004*	*0.381*	*675471*
Mbuti	***Kotias (CHG)***	***Bichon (WHG)***	***Ukraine_HG1***	*0.0054*	*0.494*	*688140*
Mbuti	***Kotias (CHG)***	***Bichon (WHG)***	***Ukraine_N1***	*0.0057*	*0.551*	*669022*

Latvia_HG, Latvian hunter-gatherers; WHG, western hunter-gatherers; EHG, eastern hunter-gatherers; CHG, Caucasus hunter-gatherers.

Tests performed using the whole genome panel are italicized, otherwise tests were performed using the Human Origin transversion SNP panel. Ancient samples are highlighted in bold. Samples include in each ancient group can be found in Supplemental Dataset 1. Key *D*-statistics for Latvian samples are shown in Table 2.

Supplemental Experimental Procedures

Archaeological information on sites, samples and their context

The Neolithic transition in the Baltic and Dnieper Rapids, Ukraine

The first evidence for the adoption of some elements of a food producing economy in the Baltic began ~6,400 cal BP, and continued over the following 5,000 years with the gradual establishment of farming communities [S7,S8]. Stable isotope analyses at Zvejnieki indicates a considerable variability across time from the late Mesolithic to the end of the Middle Neolithic, with a diet that is rich in terrestrial/freshwater proteins and of high trophic level species [S9]. There is in general a shift from a diet which relies mainly on freshwater fish during the late Mesolithic and Early Neolithic periods, to one which relies more on terrestrial animals and plants, during the Late Neolithic [S9].

Similarly at the Dnieper Rapids, Ukraine, the appearance of pottery in several of the cemeteries dates to between 7,500-7,000 cal BP but the first appearance of domesticates occurs only after 7,000 cal BP. Stable isotope analysis from cemetery populations in this region suggests variation in either the exploitation or availability of certain freshwater resources across the Mesolithic to Neolithic periods [S7].

Zvejnieki, Latvia

The site of Zvejnieki is situated in Northern Latvia, on the north-eastern bank of Lake Burtnieks. The shores of the lake have been quarried for gravel since the early 1960s and this activity led to the discovery of several prehistoric burials, some of which had traces of red ochre. This was followed by test excavations in 1964. Continuation of fieldwork during the 1960s and 1970s, covering a total of 4200 m^2, revealed 317 burials and a rich archaeological assemblage which included flint spearheads, arrows, bone harpoons, bone pendants, amber ornaments and pottery. The Zvejnieki archaeological complex consists of several groups of burials. Close to the cemetery, two settlement phases are known: the Mesolithic settlement Zvejnieki II and Neolithic settlement Zvejnieki I [S10,S11]. Six individuals from this site were selected for genetic analysis. The following information on these burials was taken from [S10–S13].

Mesolithic samples

Latvia_HG1: Burial 313. Adult female. Fragmentary skeletal material. ^{14}C date: LuS 8220, 7525 ±60 BP (8,417-8,199 cal BP).

Latvia_HG2: Burial 93. Adult male. Buried in extended supine position with head facing southwest. Grave goods included 23 teeth pendants, one beaver bone and three bird bones. Ochre layer surrounded the skeleton. ^{14}C date: Hela-1212, 6840 ±55 BP (7,791-7,586 cal BP).

Latvia_HG3: Burial 121. Adult. Described as female based on morphology but genetically determined to be male. Buried in extended supine position with head facing south. Animal teeth pendants were scattered around the burial as well as on the breast, shoulders and along the legs. Grave goods included a perforated animal phalange, two bird bones, a stone object (possibly representing an animal) and a flint chip. ^{14}C date: Ua-19883, 6145 ±80 BP (7,252-6,802 cal BP).

Neolithic samples

Latvia_MN1: Burial 124. Adult male. Buried in supine position with head facing southwest. Fragmentary skeletal material. Seven teeth pendants found in the vicinity of the head and breast. ^{14}C date: Ua-3639, 5280 ±55 BP (6,201-5,926 cal BP).

Latvia_MN2: Burial 221. Adult. Fragmentary skeletal material. Described as male based on morphology but genetically determined to be female. Part of a collective burial which contained five adults and one child. Buried in extended supine position. Heavily strewn with ochre. Grave goods included rich amber artefacts, two flint arrowheads, five flint chips, a two-headed water-bird figurine made of antler, an ornamented clay figurine and two bone fishing hook fragments. ^{14}C date: Ua-19813, 5180 ±65 BP (6,179-5,750 cal BP).

Latvia_LN1: Burial 137. Adult. Fragmentary bone material. Described as male based on morphology but genetically determined to be female. Body buried on left side, head facing east. Grave goods included a stone awl, a chisel initially thought to have been made from a deer bone but later determined to be from a domesticated goat, and potsherds. ^{14}C date: Ua-19811,4280 ±60 BP (5,039-4,626 cal BP).

Vasilyevka 3 and Vovnigi 2, Ukraine

Human remains from the cemeteries of Vasilyevka 3 (Mesolithic) and Vovnigi 2 (Neolithic) were originally studied by Konduktorova and Gokhman [S14,S15]. One individual from each graveyard was selected for genetic analysis.

Vasilyevka 3

The cemetery of Vasilyevka 3 is located on the third loess terrace of the left bank of the Dnieper river in the vicinity of Vasilyevka village (Dnipropetrovsk region). This is the biggest Mesolithic cemetery in the area. The cemetery was excavated by Telegin in 1955. 45 skeletons were found in a total of 100 m^2. It was suggested that approximately the same number of burials were destroyed by a 4 m wide gully which cut through the central part of the cemetery.

Based on the positions of the skeletons, two main burial groups were defined. The first group included 34 individuals buried in a crouched position on their right or left side, the second group included 7 individuals who were buried in supine position. The crouched burials are characteristic of Mesolithic burials from Eastern Ukraine. Most graves contained a single individual however there were also several double and triple burials.

In contrast to other Mesolithic cemeteries from the Dnieper area, for example Vasilyevka 1 and Voloshsky, where most of burials had east or south-east orientation, in Vasilyevka 3, different orientation for both main groups were found. It is possible that the heterogeneity in Vasilyevka 3 burial practices point to the introduction of a new type of funerary rite. Burials in supine position prevailed towards the end of the Mesolithic and into the Neolithic period [S16]. Artefacts found in the Vasilievka 3 burials included flint microliths, sometimes installed in bone weapons, and perforated shells.

The sample taken for genetic analyses (Skeleton 37; 14C date: Poz-83447, 9530 ±80 BP, 11,143-10,591 cal BP) was from a triple burial. All skeletons in the burial were determined to be male based on morphology. They were found in a crouched position on their left side. One skeleton in the burial had a microlith arrow point stuck in his left tenth rib. Several flint pieces were also found in his chest area. Because of this, and the very robust characteristics of all skeletons in the burial, it has been proposed that they were warriors who died and were buried simultaneously [S15]. It is believed to be one of the earliest graves in the cemetery as it was the deepest and another burial was found above it (skeleton 11). Radiocarbon dating of two crouched and one extended burial show that this graveyard was in use between 12,350 and 11,150 cal BC [S17–19].

Vovnigi 2

The Neolithic cemetery of Vovnigi 2 was excavated by Bodjanskiy and Rudinskiy from 1949 to 1952. The graveyard is located on the right bank of the Dnieper River in the very center of the village of Vovnigi (Dnipropetrovsk region, Ukraine). In the excavated area covering ~100 m^2, a total of 131 burials were excavated. It has been proposed that the large number of burials at Vovnigi, compared to earlier Neolithic cemeteries in the region, resulted from a population increase towards the end of the Neolithic [S20].

All burials were arranged in three rows with 70 burials forming the central group. Such specific cemetery organization is characteristic of the Dnieper-Donets Neolithic culture. Most skeletons were in western or north-western orientation. They were buried in supine position with their hands positioned over their pelvis or in some cases their arms were extended along their body. The skeletal remains used in our genetic analyses ((skeleton 2; 14C date: Poz-83446, 5590 ±50 BP, 6,469-6,293 cal BP) came from a single burial located within the main core of burials. This individual was buried in extended supine position in north-south orientation.

Overlapping burials were typical, especially in the central group of the cemetery. Many skeletons were covered with a thick layer of ochre. Remarkably, in all cases, burials with ochre overlay burials without it. It has therefore been proposed that the use of ochre in burial practices was developed during the later stages of the use of the cemetery [S21]. Artefacts in the burials (“grave goods”) were not numerous, consisting of small flint blades and flakes, microliths, pearl beads and shell fragments (belonging mostly to the genus *Unio*). Some of the shell fragments had artificial holes which point to their use as pendants. The presence of deer teeth and a few pottery sherds in several burials was reported. It has been suggested that the small fragments of pottery found in graves most probably came from broken pots used during funeral feasts. Most of these pottery fragments show traces of fire. The fragments were similar to pottery found at Dnieper-Donets Neolithic settlements. The Dnieper-Donets culture was spread across the western Azov Sea area, the Dnieper and the Crimean Steppes during Neolithic. Although pottery appeared in settlements of the Ukrainian Steppe zone during Early Neolithic times (8,250 cal BP), its use in funerary rituals began only 300 years later [S22]. The Vovnigi 2 site has been dated by Jacobs to 7,430 to 6,700 cal BP [S7] with the date of our sample extending the use of the cemetery by several centuries.

DNA extraction, library preparation, sequencing and alignment

In dedicated ancient DNA facilities, DNA from ancient Latvian and Ukrainian samples was extracted from (powdered) petrous bones using a silica column based protocol [S23] as described by Gamba et al., 2014 [S24]. Next-generation sequencing libraries were constructed and amplified with AccuPrime Pfx Supermix (Life Technology) following Gamba et al., 2014 ([S24]; a protocol adapted from Meyer and Kircher 2010 [S25]). Libraries were first screened to assess their human DNA content on an Illumina MiSeq platform at TrinSeq, Dublin using 50 base pair (bp) single-end sequencing. Selected libraries were further sequencing using 70 bp single-end sequencing (MiSeq platform at TrinSeq), 75 bp single-end sequencing (NextSeq platform at University of Potsdam) or 100 bp single-end sequencing (HiSeq 2,000 platform at Beijing Genomics Institute or at Macrogen).

Reads needed to be within 1 bp of their given index in order to be used in the alignment. Adapter sequences were removed from the 3’ end of reads using Cutadapt (version 1.3) [S26], requiring an overlap of at least 1 bp between the read and the adapter sequence. Reads were aligned to the GRCh37 build of the human genome with the mitochondrial sequence replaced by the revised Cambridge reference sequence (NCBI accession number NC_012920.1) using Burrows-Wheeler Aligner (BWA) version 0.7 [S27]. Sequences from the same sample, but from different sequencing runs, were merged using Picard MergeSamFiles (http://picard.sourceforge.net/) and duplicate reads were removed using SAMtools version 0.1.19 [S28]. A minimum length threshold of 30 bp was imposed and Genome Analysis Toolkit (GATK) DepthofCoverage [S29] was used to calculate the average depth of coverage. Indels were realigned using GATK RealignerTargetCreator and IndelRealigner [S29]. Sequences with a mapping quality of ≥ 30 were retained using SAMtools [S28] and 2 bp were soft-clipped from the start and end of every read.

Authenticity of data

The authenticity of data was first assessed by looking for short average sequence length as described in Gallego Llorente *et al.,* 2015 [S30]. MapDamage 2.0 [S31], with default parameters, was then used to look for and evaluate patterns of molecular damage which are typical of ancient DNA. Contamination in ancient mitochondrial sequences was estimated by assessing the number of non-consensus bases (minimum base quality threshold of 20) at haplogroup defining positions as a function of the total coverage for each of these sites [S24,S32]. X chromosome contamination levels were also evaluated for male samples with greater than 0.5-fold coverage (a cut-off recommended by Allentoft *et al.,* [S33]), using ANGSD [S34] with parameters recommended on the ANGSD website and imposing a minimum base quality threshold of 20.

SNP calling and merging with modern dataset and other ancient samples

Genotypes for modern individuals from the Human Origins dataset [S35] were merged with additional, publicly available modern genotypes (from Assyrian, French, Iranian, Italian, Lebanese, Libyan, Moroccan and Romanian populations) outlined in Lazaridis *et al.,* 2016 [S36]. Overlapping SNP positions between both datasets were retained and genotypes on sex chromosomes were removed. Using PLINK [S37], this modern dataset was merged with ancient genotypes provided in the Lazaridis *et al.,* 2016 [S36] dataset which included ancient samples described in [S24,S30,S33,S35,S38–S49]. For our Latvian and Ukrainian ancient samples, we called genotypes at positions which overlapped with this dataset using GATK Pileup [S29]. Bases were required to have a minimum quality of 20 and only alleles found in the Human Origins dataset were considered. For SNP positions with more than one base call, one allele was randomly chosen with a probability equal to the frequency of the base at that position. This allele was duplicated to form a homozygous diploid genotype which was used to represent the individual at that SNP position [S50]. This method of SNP calling was also used to call genotypes in ancient samples from Cassidy *et al.*, 2015 [S51]. These data as well as ancient data from Fu *et al.*, 2016 [S52] were merged with the modern data and other ancient samples using PLINK [S37]. For all populations genetic analyses (PCA, ADMIXTURE, *D*-statistics and f_3-statistics), transition sites were removed from the dataset in order to reduce the impact of cytosine deamination on results and only ancient samples with ≥ 15,000 called SNPs were included.

D-statistics were also performed on a panel of whole genome sequences which contained both modern [S38] and ancient genomes (samples from this study along with Bichon, Kotias [S45], NE1, BR2 [S24], Mal'ta [S38], Loschbour, Stuttgart [S35] and Karelia [S52]). Ancient genomes were aligned, filtered and pseudo-diploid genotypes called as described above, imposing a minimum base quality threshold of 30. Modern genotypes were also made pseudo-diploid by choosing one allele at random and duplicating it. Only autosomal transversion SNPs found in the Phase 1 of the 1,000 Genomes Project were used in these analyses.

Population genetic analyses

PCA was performed using EIGENSOFT 5.0.1 smartpca [S53] to project ancient data onto the first two principal components defined by a Eurasian subset of our modern dataset. This was carried out with the lsqproject option on and the outlier removal option off. One SNP from each pair in linkage disequilibrium with $r^2>0.2$ was removed. ADMIXTURE version 1.23 [S54] was used to perform a clustering analysis on the dataset described above. Single-nucleotide polymorphisms in linkage disequilibrium were thinned using PLINK (v1.07) [S37] with parameters --indep-pairwise 200 25 0.5 [S43]. Clusters (*K*) (2–20) were explored using 10 runs with fivefold cross-validation at each *K* using different random seeds. The minimal cross validation error was found at *K*=17 (see Figure S6; results for *K*2-20 are shown in Figure S5 and Figure S7 for modern and ancient individuals respectively). *D*-statistics [S55] and f_3-statistics [S56,S57] were computed using the qpDstat and 3PopTest programs respectively from the ADMIXTOOLS package [S57].

Uniparental haplogroups

For mitochondrial and Y chromosome haplogroup determination, sequences were rescaled using mapDamage 2.0 [S31] to reduce the impact of terminal sequence deamination on results. ANGSD [S34] was used to create mitochondrial consensus sequences setting the minimum depth of coverage to 3 and the minimum base quality to 20. Haplogroups were then determined using the HAPLOFIND [S58] web-tool which classifies mitochondrial sequences according to previously annotated haplogroups. YFitter [S59] was used to determine the Y chromosomal haplogroups of our male samples. Genotypes at positions along the Y-chromosome which overlapped with the dbSNP (build 137) database were called in male samples using GATK [S29]. VCFtools [S60] was used to convert the resulting VCF files to PLINK format files [S37] which were subsequently converted to qcall format using the tped2qcall.py script provided in the YFitter package. Yfitter was run with default parameters using the build 37 Y chromosome tree outlined in Karafet *et al.*, 2008 [S61].

Genotypes associated with particular phenotypes

For selected markers, genotypes with alleles present in Phase 1 of 1,000 genomes dataset [S62] and with base quality ≥ 20, were called using GATK Unified genotyper [S29]. As many diagnostic markers were not sequenced or had low coverage, we used imputation to infer genotypes at these positions. Imputation was performed as described in Gamba *et al.*, 2014 [S24], imputing at least 1 Mb upstream and downstream of the SNP position where possible and using 10 iterations to estimate genotypes at ungenotyped markers. The Hirisplex prediction model [S6] was used to explore hair and eye colour with a genotype probability threshold of ≥ 0.7 imposed.

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