

Harnessing haplotype sharing information from low coverage sequencing and sparsely genotyped data

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I, Sam Morris, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the work.

Abstract

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Chapter 1

Introduction

1.1 Chromopainter and ancient DNA

In this introduction I will outline the following: i) What are ‘haplotype-based’ methods and what advantages and disadvantages do they offer over ‘unlinked’ methods, ii) a summary of different methods used to analyse ancient DNA and iii) the need to merge datasets genotyped on different arrays and options for imputation.

1.1.1 Gains to be made with haplotype information

Haplotype-based methods are those which explicitly model Linkage Disequilibrium (LD) between neighbouring SNPs along a haplotype. This is in contrast to ‘unlinked’ methods, which assume a model of Linkage Equilibrium between SNPs. A ‘haplotype’ is a sequence of alleles along chromosome. Note that other methods, for example `octopus` [1] are referred to as ‘haplotype-based’ genotype callers, but they represent a distinct group of methods to e.g. ChromoPainter.

Linkage Disequilibrium (LD), the non-independence of alleles carried at different positions in the genome, has been studied since the earliest days of genetics [2,3] and has since been a fundamental aspect of virtually all areas

of genetics. In this thesis, here I will focus on its application to inference of haplotype sharing between individuals.

The history of using LD to inform of human population history began in... Early studies leveraged the information present in LD patterns at the CD4 locus to show the preferred model of Human population history was a recent African origin [4]. Further studies 2000s [5] utilised the then-new Hap-Map results to show LD varies markedly across worldwide populations [5], and although the study only used a small number of SNPs ($n=3024$) relative to the number used today, it revealed insights which are still relevant today, such as the presence of long haplotype blocks in highly drifted Native American populations.

Under the most basic model of random mating and a constant population size, individuals who share recent ancestry are expected to share longer regions of alleles which are identical-by-descent (IBD) than those who do not. As recombination breaks haplotypes across generations. This means haplotype-based methods are especially powerful at detecting recent shared ancestry and admixture events within the last 500 years.

Accounting for recombination and LD within a model is necessarily computationally complex, as the number of combinations of alleles and their possible evolutionary histories balloons as the number of loci considered increases (does it scale quadratically?). The development of the Li and Stephens copying model (LSM) [6] was instrumental in the development of such methods [7] and provided an elegant solution to the increased complexity when using linked loci. As such, it is now a critical model in virtually all areas of genomic methodology, such as gene conversion parameters, admixed populations, human colonization history, local ancestry in admixed populations, imputation and haplotype estimation.

Perhaps the first paper to formalise haplotype-based approach for the study of population history was that of Hellenthal et al 2008 [8]. Building on the copying model proposed by Hellenthal et al, Lawson et al (2015) [9] created

ChromoPainter, a model which explicitly accounts for linkage and variable recombination rates in order to find the genealogically closest haplotype to a target in a set of references. The authors showed that ChromoPainter had an enhanced ability to separate closely related populations when plotted on a PCA compared to unlinked methods. ChromoPainter was originally developed in tandem with its own clustering method fineSTRUCTURE, and has since been extended into methods to detect and date admixture [10], and infer ancestry proportions [11].

ChromoPainter can be run in either ‘linked’ or ‘unlinked’ mode. In the linked mode, described in detail in later sections, LD between neighbouring SNPs is accounted for. ‘Unlinked’ mode assumes a model of linkage equilibrium between markers and has been shown to be statistically identical to the likelihood model underlying the commonly used ADMIXTURE algorithm.

A typical case study, and one which I will return to in later chapters, was a study which attempted to identify population structure among individuals from the British Isles. This study, hereafter referred to as POBI, genotyped 2039 people from England, Wales and Scotland. In summary, it was possible to detect structure down to the level of Devon and Cornwall (two neighbouring counties) using ChromoPainter. On the other hand, little structure was apparent when using unlinked methods (PCA). This outlines the benefits of incorporating linkage information when attempting to identify fine-scale structure between closely related populations.

It has since become a mainstay of population genetic research, both in humans and other organisms. It has also been incorporated into many ancient DNA studies, which I will discuss in the next section.

However, the usage of haplotype-based methods is not without drawbacks. They are typically slower by several orders of magnitude, as the computational complexity is something.

Secondly, the nature of haplotype-based methods means they require the data to be phased. Phasing is a statistical procedure¹ that requires substantial computation resources. Phasing is a procedure which is often error-prone (switch errors).

1.2 Methods used to analyse ancient DNA

The first ancient DNA papers mostly relied on statistical methods which compare allele-sharing or allele-frequencies between populations or individuals. These methods, in particular f-statistics and their extensions [12–14] and Principle Component Analysis [15], can address a wide-range of questions pertaining, but not limited to, population structure, admixture, genetic similarity and population graphs.

The early studies of ancient DNA generally considered broad-scale questions about human history, such as the nature of human-archaic interactions or the spread of farming technology across Europe [16]. Due to the infancy of the field, sample sizes were small, as efficient methods to obtain genetic data from ancient samples, such as SNP capture arrays, had yet to be developed. Early studies tended to look for genetic differences between populations which diverged many generations ago. For example, in the case of Lazaridis et al (2014), simply plotting Loschbour and Stuttgart on a PCA of modern individual showed they had substantially different ancestries, with Loschbour being most similar to present-day North-East Europeans, but falling well outside the variation of present-day Europeans, and Stuttgart clustering with present-day individuals from Tuscany.

Such methods, which I will hereafter refer to as ‘unlinked’, as they assume a model of linkage equilibrium (i.e. each SNP is independent of another in the context of) between neighbouring SNPs, are useful for ancient DNA studies.

¹Phasing can also be performed using other methods, such as sequencing family trios. However, this is rarely used in population genetic studies and so I will not discuss it here

Data is often represented in ‘pseudo-haploid’ format to avoid making diploid genotype calls at low coverage positions; true heterozygous positions may be mis-called as homozygous at low coverages, as there is a probability of $p()$ that no reads mapping to a particular allele may be sampled. Representing variants pseudo-haploid is used widely in past and current ancient DNA studies. It is also simple to account for missing data in PCA and f-statistics.

However, in recent years, the low hanging fruit of broad-scale questions have mostly been answered and studies into more fine-scale populations structures have become more prevalent. Accordingly, methods which can detect more subtle population structure have been required. Include example here.

Another reason for the use of alternate methods to f_3 statistics is that they possibly display bias in the face of drifted populations.

The first use of chromopainter on ancient DNA was in a seminal paper from Lazaridis et al (2014) [16]. The two samples, Loschbour and Stuttgart, were of high coverage and therefore imputation was not used.

The first study to explicitly investigate the reliability of ChromoPainter on ancient DNA was Martiniano et al (2017) [17]. They first tested the accuracy of imputation on ancient DNA samples by downsampling high coverage ancient genomes and comparing imputed to full-coverage non-imputed genotypes. Overall, they found a good correspondence. They also found that filtering the SNPs based on posterior genotype probabilities had little effect.

As there is currently no way to allow missing positions ChromoPainter, imputation is necessary. Recently, Hui et al (2020) evaluated the accuracy of genotype imputation in ancient DNA samples [18].

As sample sizes used in ancient DNA studies has rapidly increased to >100 , more analyses have incorporate haplotype-based methods. Each study typically carries out a small analysis to ensure that imputation in low-coverage

ancient DNA samples is accurate. Antonio et al (2019) [19] analysed 127 ancient genomes of a mean coverage of 1x. To test imputation accuracy, they downsampled a single individual (NE1) to different levels of coverage and calculated the proportion of genotypes which matched the full coverage. However, this analysis was only performed on a single sample and the effect of imputation on the chromopainter process was not evaluated. As of writing (September 2021), the study of Margaryan et al (2020) is the biggest so far to use ChromoPainter, with over 400 samples used [20].

More recently, ChromoPainter has been used to study aspects of archaic hominin ancestry in present-day humans [21, 22].

1.3 Issues with low coverage

Coverage is an issue which has plagued the field of ancient DNA since its inception. Compared to DNA obtained from present-day samples, ancient DNA samples typically have a much lower proportion of endogenous DNA. This is because DNA degrades over time from environmental factors. Therefore, when the DNA fragments are sequenced, relatively few of them will align to the human reference. The coverage of a genome is therefore the mean number of reads mapped to each position in the genome.

The primary issue with low-coverage data is the increased uncertainty when calling diploid genotypes, particularly when the true genotype is heterozygous. To mitigate this issue, many studies used ‘pseudo-haploid’ genotyping, where a diploid genotype is reduced to a haploid by randomly sampling an allele from all the reads which have been aligned to that position in the genome. Whilst the use of pseudo-haploid calling eradicates the need to call heterozygous genotypes at low coverage positions, it also necessarily reduces the amount of information present at each position in the genome.

I will discuss in more detail later the effect of coverage on other methods.

1.4 Combining data from multiple chips

A related issue stems from the current practice of developing a large number of genotyping arrays. Different cohorts are genotyped on different arrays and sets of SNPs, as different SNPs have different characteristics. For example, some SNPs are known to be associated with particular phenotypes, some SNPs are known to be more variable (and therefore more informative at identifying structure) in certain populations. Whilst this generation of custom genotyping arrays has meant a wider variety of questions and populations can be studied using genotyping arrays, it also makes combining data from across different arrays potentially troublesome, as they often have a small overlap in the SNPs upon which they have been genotyped.

For example, in my thesis, I have worked with at least 3 genotyping arrays; ‘Human Origins’, ‘Hell Bus’ and the UK Biobank. Often I have wanted to compare populations on different arrays, such as the African populations on the Human Origins array and UK Biobank individuals on the UK Biobank array. After merging the datasets, the overlap was small, only 70,000 SNPs. This is around an order of magnitude fewer SNPs than a typical ChromoPainter analysis.

Having a smaller number of SNPs may reduce power in two ways. Firstly, there is simply fewer informative data points to use when comparing the SNP patterns between two populations and therefore fewer possible data points which can be used to identify populations. Secondly, ChromoPainter derives part of its power from the LD between neighbouring SNPs. LD between two neighbouring SNPs is correlated with their physical distance. Fewer overall SNPs means each neighbouring pair of SNPs are physically further away from one another and thus have less LD information.

One solution to the issue of a small number of SNP would be to impute the remaining SNPs. In this context, imputation refers to estimating missing

genotypes using of a model usually based upon the LSM and a large reference panel. Imputation is widely used in e.g. GWAS studies to generate sequence-level data.

However, it is possible that imputation may cause a bias in the data. If missing genotypes are imputed incorrectly more often from one population than another, this will result in an increased, but spurious genetic similarity between the target and reference population. This may be a particular issue when analysing populations which are not well represented in imputation reference panels, such as non-Europeans. The nature and magnitude of this bias, however, is yet to be fully understood, particularly in the context of ChromoPainter.

Therefore, one question to ask is the following; is it more desirable to impute the missing positions or to use a smaller number of overlapping SNPs. This is something which I will investigate in chapter 3 with a case study investigating African ancestry in the UK Biobank dataset.

Chapter 2

ChromoPainter and ancient DNA

2.1 Introduction

This chapter is related to the use of ChromoPainter on low coverage ancient DNA samples.

First, I will describe the existing methodology, ChromoPainterV2, and then two new versions, ChromoPainterUncertainty and ChromoPainterUncertaintyRemoveRegions, which are designed to attempt to mitigate bias related to sequencing coverage.

Next I will perform benchmarking tests on all the steps necessary to analyse low-coverage ancient DNA with ChromoPainter. This includes genotype calling and genotype likelihood estimation with atlas [23], phasing and genotype imputation with GLIMPSE [24], ChromoPainter [9] analysis (copy-vector estimation and PCA) and SOURCEFIND ancestry component estimation [11]. Lastly, I will describe some of the existing issues pertaining to low coverage ancient DNA and several considered mitigation strategies.

2.2 Methods

2.2.1 Description of the ChromoPainter algorithm

ChromoPainter is a method designed to infer patterns of haplotype sharing between individuals [9]. The individuals being analysed are split into ‘donor’ and ‘recipient’ haplotypes. An individual may or may not be both a donor and recipient, but they cannot act as a donor to themselves. In diploid organisms such as humans and dogs, each individual thus consists of 2 haplotypes. It employs the widely-used Li and Stephens copying model [6] to model each recipient haplotype as a mosaic of haplotypes observed in the donor panel. Unlike the original Li and Stephens model, which uses the product of approximate conditionals (PAC likelihoods), ChromoPainter reconstructs each recipient haplotype as a mosaic of *all* other donor haplotypes. Here, the term ‘copying’ can be thought of as a genealogical process where haplotypes are reconstructed using the genealogically closest haplotype. The copying model is implemented in the form of a Hidden Markov Model (HMM), with the observed states being the genotype data, and the hidden states being the ‘nearest-neighbor’ haplotype the recipient haplotype copies from. The emission probabilities are given as the probability of a recipient haplotype copying from a particular donor haplotype, given their respective genotypes. Consider a donor d and recipient r , each with an allele x at position p . There are two possibilities - either the alleles match between the donor and recipient at p , or they do not. The probability of r copying from d is:

$$\Pr(r = x \mid d = x) = [(1 - \theta) * z_{dr}] + [\theta * z_{!dr}] \quad (2.1)$$

where $z_{dr} = 1$ if d and r both carry allele x , and otherwise $z_{!dr} = 0$, and θ is some pre-specified error likelihood, set to default as 0.001.

The transition probabilities (i.e. the probabilities of a change in r copying

from one donor haplotype to another) is guided by a recombination rate map, with higher recombination rates leading to a higher probability of transitioning. Switches between donors are interpreted as changes in ancestral relationships because of historical recombination.

In ChromoPainterV2, the input genetic data comes in the form of genotype calls (i.e. 1/0, A/T/C/G).

ChromoPainterV2 produces several different output files. The two which are most used in this work are those appended with .chunklengths and .chunkcounts. In the chunklengths matrix, cl , the entry $cl_{d,r}$ gives the total number of chunks that recipient r copies from donor d . Thus, higher values of $cl_{d,r}$ indicate that recipient r and donor d share more recent ancestry.

In this work, 'copyvector' is used to refer to the vector of chunklengths that a single recipient individual copies from all donors.

2.2.1.1 Description of ChromoPainterV2Uncertainty

ChromoPainterUncertainty works in a very similar way to ChromoPainterV2, bar two differences. Firstly, the input data is in the form of an allele probability $0 \leq x \leq 1$, which is given as the probability of observing the alternate allele at that position in the genome. This value is calculated from the posterior likelihood that an allele has been imputed correctly. This is different to ChromoPainterV2, which uses 'hard' allele calls that can only take a value of 0 or 1.

Consider the following example: we have a phased genotype in the form 0|1, corresponding to the reference allele on the first haplotype and the alternative allele at the second haplotype. I define G as the sum of the genotypes at a SNP; in this case $G = 0 + 1$.

We also have a posterior genotype likelihood, in the form $GL(p_a, p_b, p_c)$,

where p_a , p_b , and p_c are the posterior genotype probabilities for each of the 3 possible genotypes. Dosage, D , is the expected total number of copies of the alternate allele given GL . D can be calculated as $p_b + [2 * p_c]$. We can calculate U , the uncertainty as $U = |G - D|$. Intuitively, U gives the difference between the observed and expected dosages, with larger differences corresponding to a higher value of U . Then, we can assign a probability to each allele; if the allele is 1 then the allele likelihood is simply $1 - U$ and if the allele is 0 then the allele likelihood is $0 + U$.

The second difference is the incorporation of the allele probability into the emission probability of the HMM. As before, consider a donor d and recipient r at position p .

$$\begin{aligned} p(r_x|d_x) = & (1 - \theta) * [r_x * d_x + (1 - r_x) * (1 - d_x)] \\ & + \theta * [r_x * (1 - d_x) + (1 - r_x) * d_{xp}] \end{aligned} \quad (2.2)$$

, where r_x is the probability that r carries the alternate allele, and d_x is the probability that the donor carries the alternate allele. Note that above (3) reduces (1) if $d_x = 0/1$ and if $r_x = 0/1$ (i.e. there is no uncertainty in the calls).

2.2.2 Generation of downsampled genomes

I created a set of ‘downsampled’ ancient genomes in order to explicitly quantify the effect of coverage at each stage of the ChromoPainter analysis. I took several high coverage genomes and for each, removed a random subset of reads from the .bam file in order to reduce the coverage to a target level. I then performed each stage of a typical ChromoPainter analysis (for example, on a novel set of ancient DNA described in chapters 4 and 5) on the full coverage and downsampled genomes, and compared the results to determine how they are affected by coverage.

Five high coverage ancient genomes were downloaded in the form of aligned .bam files from the European Nucleotide Archive: (1) Yamnaya (Yamnaya Bronze Age steppe-pastoralist) [25], (2) UstIshim (Siberian Upper Paleolithic hunter-gatherer) [26], (3) sf12 (Scandinavian Hunter-Gatherer) [27], (4) LBK (early European farmer from the Linearbandkeramik culture from Stuttgart, Germany) [16] and (5) Loschbour (an 8,000 year-old hunter-gatherer from Luxembourg) [16]. These samples were chosen due to their high original coverage because they are a diverse representation of ancestries present in Europe over the past 40,000 years.

Each original full-coverage .bam file was processed using the atlas (version 1.0, commit f612f28) pipeline [23] (<https://bitbucket.org/wegmannlab/atlas/wiki/Home>). First, each file was validated using ValidateSamFile command from PicardTools [28].

I then downsampled each full-coverage genome using the `atlas downsample` task, resulting in a .bam file with coverages 0.1x, 0.5x, 0.8x, 1x, 2x, 3.5x, 5x, 10x and 20x per individual.

For each full coverage and downsampled .bam file, I estimated post-mortem damage (PMD) patterns using the `atlas estimatePMD` task. Recalibration parameters were then estimated using the `atlas recal` task. Finally, both the recalibration and PMD parameters were given to the `atlas callNEW` task which produces genotype calls and genotype likelihood estimates for each downsampled and full coverage .bam. For this stage, I made calls at the 77,818,345 genome-wide positions present in the phase 3 thousand genomes project [29]. This was done to reduce the risk of calling false-positive non-polymorphic genotypes.

2.2.3 Generation of ancient samples

I also generated a set of ancient samples to use as donors in the ChromoPainter analysis (Appendix table 1).

This dataset consists of 918 other ancient samples from the literature. These samples were of variable coverage and chosen because they were relevant to studying the cultures discussed in chapters 4 and 5. These 918 consist of all samples from appendices A1, A2, A3, A4, and they were processed in an identical way to the downsampled target individuals described in the previous section, other than they were not downsampled.

2.2.4 Imputation and phasing - GLIMPSE

Genotype imputation and phasing are two important steps for processing low-coverage ancient DNA. Low coverage (<1x) samples typically lack enough read information to make accurate genotype calls at most positions in the genome, or may not contain any information at some sites at all [30]. Therefore, it can be helpful to use external information from a reference panel in order to improve the accuracy of genotype calls and reduce the impact of errors on downstream analyses [24]. Given ChromoPainter uses haplotypes rather than genotype data, it is also necessary to phase the genotypes. Phasing refers to the process of determining which alleles were inherited together on the same chromosome. Imputation and phasing must be performed on all full coverage, downsampled individuals.

Three different characteristics are desirable for an imputation algorithm to be useful in this context. Firstly, to allow an input in the form of genotype likelihoods. This is because genotype likelihoods allow for flexible representation of the possible genotypes at a particular position, particularly when there may not be enough coverage to make a hard genotype call. Secondly, to emit posterior genotype-probabilities which, when accurately calibrated, give the

probability that a particular genotype call is correct. This is crucial for our previous step 2.2.1.2 for including these genotype probabilities into the painting process. Thirdly, the algorithm must be able to complete in a reasonable running time when using a large number of samples and high number of SNPs. Using a large number of densely positioned SNPs (e.g. such as the approximately 77 million identified in the 1000 genomes project) increases the useful linkage-disequilibrium information between each SNP, and it is well-known that increasing the number of individuals used in imputation/phasing reference panels improves accuracy [24, 31–33].

Two programs, Beagle 4.0 [34] and GLIMPSE [24] fulfill the first and second criteria above, but only GLIMPSE runs quickly enough to analyse SNPs with sequence-level density. GLIMPSE offers up to 1000x reduction in running time compared to Beagle 4.0 [24], so I chose to use this algorithm for the imputation and phasing steps.

Phasing and imputation ideally requires a reference panel of high-coverage present-day individuals. I used the 1000 Genomes dataset (re-sequenced to 30x coverage), containing 3202 individuals from 26 worldwide populations [35]. A description of the processing of this reference dataset can be found in appendix

...

I next merged together i) the full coverage individuals, ii) downsampled individuals and iii) 918 ancient samples from the literature into a single bcf file using bcftools (version 1.11-60-g09dca3e) [36] to act as the samples for GLIMPSE to phase. Here, ‘target’ refers to the individuals being imputed/phased and ‘reference’ refers to the reference panel.

Following the glimpse tutorial (https://odelaneau.github.io/GLIMPSE/tutorial_b38.html), I first used `GLIMPSE_chunk` to split up each chromosome into chunks, keeping both `-window-size` and `-buffer-size` to 2,000,000, their default settings. I used the b37 genetic map supplied by GLIMPSE for the `-map`

argument. Across all chromosomes, this produced 936 chunks of an average 2.99Mb long.

GLIMPSE then imputed each chunk separately, using `GLIMPSE_phase` using the same 1000 genomes dataset as a reference and default settings. This stage both imputes missing genotypes and generates a set of haplotype pairs which can be sampled from in a later step to produce phased haplotypes. `GLIMPSE_ligate` then merges the imputed chunks back to form single chromosomes using the default settings. I then used `GLIMPSE_sample` to produce a .vcf with phased haplotypes sampled for each individual, again using default settings. Consequently, the output of GLIMPSE is i) unphased genotype calls with posterior genotype likelihoods and ii) phased haplotypes.

It is important to note that GLIMPSE leverages information from individuals that have been imputed, ‘absorbing’ them into the reference panel. For example, if there were 100 target samples and 1000 reference samples, each target is phased in turn and then absorbed into the reference panel, so that there would be 1001 reference samples when the 2nd target individual is imputed. This makes it necessary to avoid including the same sample, downsampled to different coverages, in the same set of targets for one imputation run, in order to avoid the confounding effect of allowing an individual to act as the reference to itself. For example, including Loschbour at 0.1x and 10x coverage could mean it imputed itself, a situation which would never occur in reality.

2.2.5 Estimating imputation sensitivity and specificity

I used rtg-tools-3.11 [37] and the `vcfeval` task to estimate the sensitivity and specificity of variant discovery in the downsampled individuals. Here, ‘baseline’ (i.e. the truthset) is defined as the genotype calls in the full coverage individual and the ‘calls’ as the genotype calls in the downsampled individual. Sensitivity and precision are defined as:

$$sensitivity = \frac{TP_{baseline}}{TP_{baseline} + FN} \quad (2.3)$$

$$precision = \frac{TP_{call}}{TP_{call} + FP} \quad (2.4)$$

A ‘variant’ is considered to be a SNP with a genotype that is either 0/1 or 1/1, with $TP_{baseline}$ and TP_{call} the number of variants called in the full coverage and downsampled genomes, respectively. False negatives (FN) are where a variant is called in the full coverage genome but not in the downsampled genome. False positives (FP) are cases where a variant is called in the downsampled genome but not in the full-coverage genome.

TP , or true-positive, is the number of events where a variant position (i.e. a SNP with a genotype that is either 0/1 or 1/1) is detected in either the full coverage ($TP_{baseline}$) or downsampled ($TP_{baseline}$) sample. FN is the number of times that a variant position is called in the full coverage sample and not the downsampled sample. Conversely, FP is the number of times a variant position is called in the downsampled sample and where the same SNP in the full coverage sample is invariant (i.e. 0/0).

2.2.6 ChromoPainter analysis

It is important to understand the effect of sequencing coverage on the accuracy of ChromoPainter copyvector estimation. A ‘copyvector’, c_r , is a vector of length D , where each entry gives the total length of genome that recipient individual r most closely matches to each of the D donor individual/populations. I sometimes refer to ‘normalised’ copyvectors; this simply refers to where each entry of c_r is divided by the sum of all entries, scaling the copyvector to sum to 1.

I painted each downsampled and full coverage ancient individual using

a set of 124 ancient individuals, hereafter referred to as the ‘standard set’, selected because they had a sequencing depth greater than 2x. I compared the copyvectors for the same individual at each level of downsampling. For example, I compared the copyvector of Yamnaya at 0.1x to the copyvector of the same Yamnaya sample at full coverage. A high correspondence, measured by r-squared for example, between the copyvectors of the full coverage and downsampled individual suggests less effect of coverage.

To prepare the data for ChromoPainter, I merged the .vcf containing the posterior genotype likelihoods of i) downsampled, ii) full coverage and iii) 918 ancient samples from the literature together, and did the same for the .vcfs containing the phased haplotypes. I combined the posterior genotype likelihoods with the phased alleles to generate allele likelihoods (described in section 2.2.1.2) in ChromoPainter-uncertainty format, in addition to per-position recombination rate files. This was performed for each chromosome in turn using my own script (https://github.com/sahwa/vcf_to_ChromoPainter).

I next used ChromoPainterUncertainty to perform the painting. I assigned the ‘standard set’ individuals as donors and all downsampled, full coverage and ancient samples downloaded from the literature as recipients. This produces a chunklengths matrix for each chromosome which were merged using chromocombine-0.0.4 (<https://people.maths.bris.ac.uk/~madjl/finestructure-old/chromocombine.html>). The resulting chunklengths matrix thus gives the total length of genome in centimorgans that a recipient most closely matches to each donor individual.

2.2.7 ChromoPainter Principle Component Analysis

Principle Component Analysis (PCA) can be used to reduce the underlying structure in the chunklengths coancestry matrix to two dimensions, thus allowing it to be more easily visualised.

Principle component analysis was performed on the chunklengths matrix using the IRLBA R library [38].

2.2.8 SOURCEFIND

The chunklengths coancestry matrix produced by ChromoPainter contains information about the estimated length of genome a recipient most closely matches a given donor individual or population. However, incomplete lineage sorting, where alleles segregate in a way that is discordant to the ‘true’ phylogeny reflecting the orders in which populations split from one another, means that there are regions in the genome where a recipient individual most closely matches a reference individual that is not from the population that has split most recently from according to the ‘true’ phylogeny. This manifests itself as ‘noise’ in the co-ancestry matrix, where, for example, an individual from France copies non-zero amounts from African donors, despite not having any recent African ancestry through admixture. Furthermore, unequal donor population sizes may bias the aggregated amount copied to a given population.

Therefore, in order to account for differences in donor group size and to improve resolution in directly estimating ancestry proportions, it is necessary to run an additional step, SOURCEFIND [11]. Simulations have shown that SOURCEFIND ancestry proportions correspond well to simulated values. The ancestry proportions produced by SOURCEFIND should be interpreted as the proportion of ancestry that each individual/population shares most recently with each surrogate. This need not necessarily imply an admixture even; for instance, you might expect *France* to have ancestry recently related to both *Germany* and *Spain*, due to isolation-by-distance rather than admixture.

SOURCEFIND takes as input i) the chunklengths matrix described in section 2.2.6 and a parameter specification file. This input file allows for the definition of ‘surrogate’ individuals/populations. SOURCEFIND models each target copyvector as a linear mixture of copyvectors from the surrogate groups,

inferring the proportion of ancestry that each surrogate group contributes to the target individual. The parameter space of surrogate ancestry proportions is explored using a Markov chain Monte Carlo algorithm, where the ancestry proportions are updated using a Metropolis-Hastings step. The output of SOURCEFIND for each target individual is therefore an $n * p$ matrix, where n is the number of MCMC samples and p is the total number of surrogate groups. Ancestry proportions, credible intervals and chain mixing/convergence checking for each surrogate group were estimated using the CODA R library [39].

To test for the effect of coverage on the proportions estimated by SOURCEFIND, I performed two separate analyses, both using the down-sampled and full coverage individuals as targets. The first uses three surrogate populations (Yamnaya, Western Hunter-Gatherer and Anatolia Neolithic Farmer), and the second uses an expanded list of 37 surrogate populations (individuals and population labels in Appendix B.x). I chose the first set of three surrogates, as these are typically used in ancient DNA analysis to obtain a 'broad' overview of the ancestry of a European individual, as it has been shown that central Europeans within the last 10,000 years can be well modeled as a mixture of those three groups [16, 40]. Note, this does not mean that there was not admixture from other sources, but that a majority of ancestry of ancient central Europeans can be derived from these sources. This stands to act as a relatively 'easy' test case, since the 3 populations are highly genetically differentiated from one another.

For all runs of SOURCEFIND, I used 2,000,000 iterations, of which 50,000 were designated as burn-ins, and then samples were taken every 50 iterations. 2,000,000 iterations were chosen because my previous tests show that is the minimum necessary to provide reasonable confidence of convergence within reasonably running time (reference to appendix? **[YES, YOU SHOULD SHOW THIS.]**). The rest of the parameters were left as default. Ancestry proportions were estimated by taking the mean proportion across all iterations.

2.3 Reducing SNP count

One way to mitigate coverage-related bias would be to exclude imputed SNPs which have a low probability of being imputed correctly or restricting analysis to non-imputed SNPs above a certain coverage.

However, reducing the total number and or density of SNPs used in a painting may reduce the accuracy of the estimated copyvectors. All other things being equal, there is less linkage information between two SNPs with are separated by a larger genetic distance. Therefore, it is necessary to precisely determine what effect reducing the number of SNPs has. In particular, we would like to know the minimum number and density of SNPs required to retain the advantages of haplotype-based methods over unlinked methods.

Previous studies showed it is possible to distinguish between individuals from Devon and Cornwall using the fineSTRUCTURE algorithm, but not unlinked methods (ADMIXTURE [41]) [42]. fineSTRUCTURE mostly separated individuals from Devon and Cornwall into different clusters. Therefore, determining whether it is possible to distinguish between individuals from Devon and Cornwall acts as a good test case for reducing SNPs; how many SNPs can we remove before we lose the ability to distinguish between these two populations. Previous studies showed that it is possible to distinguish between individuals from Devon and Cornwall using ChromoPainter in linked mode, but not using unlinked methods (PCA, ADMIXTURE, ChromoPainter unlinked). Therefore, attempting to distinguish individuals from Devon and Cornwall is a useful test of whether there is still a benefit to be gained from using haplotype information.

I used the People of the British Isles (POBI) as a dataset to test this with. The original POBI dataset contains 2039 individuals from 33 populations from across England, Wales and Scotland, genotyped at 452 592 SNPs. Details of the data preparation for this dataset can be found in appendix xx.

I reduced the total number of SNPs down to a set of target levels, retaining a percentage of the original total 0.2%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%. SNPs were randomly subsetted using the `shuf` unix command. SNPs were removed from the `.vcf` files using `bcftools -view`.

For each target level of reduced SNPs, I painted all individuals from Devon and Cornwall using all 2039 POBI individuals as donors. I then combined the resulting chunklengths matrices across all chromosomes and combined copyvectors columns into donor groups.

2.4 Direct imputation test

To explicitly test the effect of imputation on the copyvectors estimated by ChromoPainter, I created a dataset which simulated a typical imputation scenario, when for example, imputing SNPs after merging two datasets with a low SNP overlap.

I took the Human Origins dataset (appendix A.19), containing 560,240 bi-allelic SNPs and selected 70,000 random SNPs to retain, removing all others using `bcftools`. I then submitted the reduced dataset to the Sanger Imputation Service (<https://www.sanger.ac.uk/tool/sanger-imputation-service/>). The Sanger Imputation Service uses Eagle2 [43] and the Haplotype Reference Consortium as a reference to impute missing variants. Once the data had been imputed, I subsetted the data back to the original set of 560,240 SNPs. I therefore had a dataset which contained 70,000 non-imputed SNPs and 490,240 imputed SNPs. This is hereafter referred to as the ‘imputed dataset’. 70,000 non-imputed SNPs was chosen because that is the number of SNPs which overlap between two datasets in Chapter 3 and thus represents a realistic case-study.

For both the imputed dataset and original Human Origins dataset, I per-

formed an all-v-all painting and combined data across chromosomes. An ‘all-v-all’ painting is where each individual is painted in turn by all other individuals, resulting in an n -by- n coancestry matrix, where n is the number of individuals analysed.

2.5 Results

2.5.1 Imputation accuracy

To estimate how accurately genotypes can be imputed in ancient samples of differing coverages, I estimated the sensitivity (Fig. 2.1) and precision (Fig. 2.2) of genotype imputation using rtg-tools [37]. This approach compares genotype calls at each position in each downsampled individual after imputation to the same individual at full coverage without imputation.

As expected, both the overall sensitivity and precision of imputation fell with coverage, with a particularly sharp drop-off in both metrics between 0.5x and 0.1x coverage.

Different downsampled individuals differed in the precision and sensitivity of genotype imputation. At all coverages, Yamnaya had the both the highest sensitivity and precision. This may be because the imputation reference panel contained more individuals who are genetically closer to Yamnaya relative to the other ancient individuals. This is consistent with present-day Europeans containing a high proportion of Yamnaya-like ancestry, relative to e.g. Hunter Gatherer ancestry [44]. Many studies in present-day individuals have shown that imputation accuracy increases when more haplotypes which are close to the target individual are found in the reference panel [31, 32]. On the other hand, the sample Ust’Ishim is known to have contributed very little genetic ancestry to present-day populations [45] and may therefore have fewer closely matching haplotypes in the reference panel, and a correspondingly lower imputation

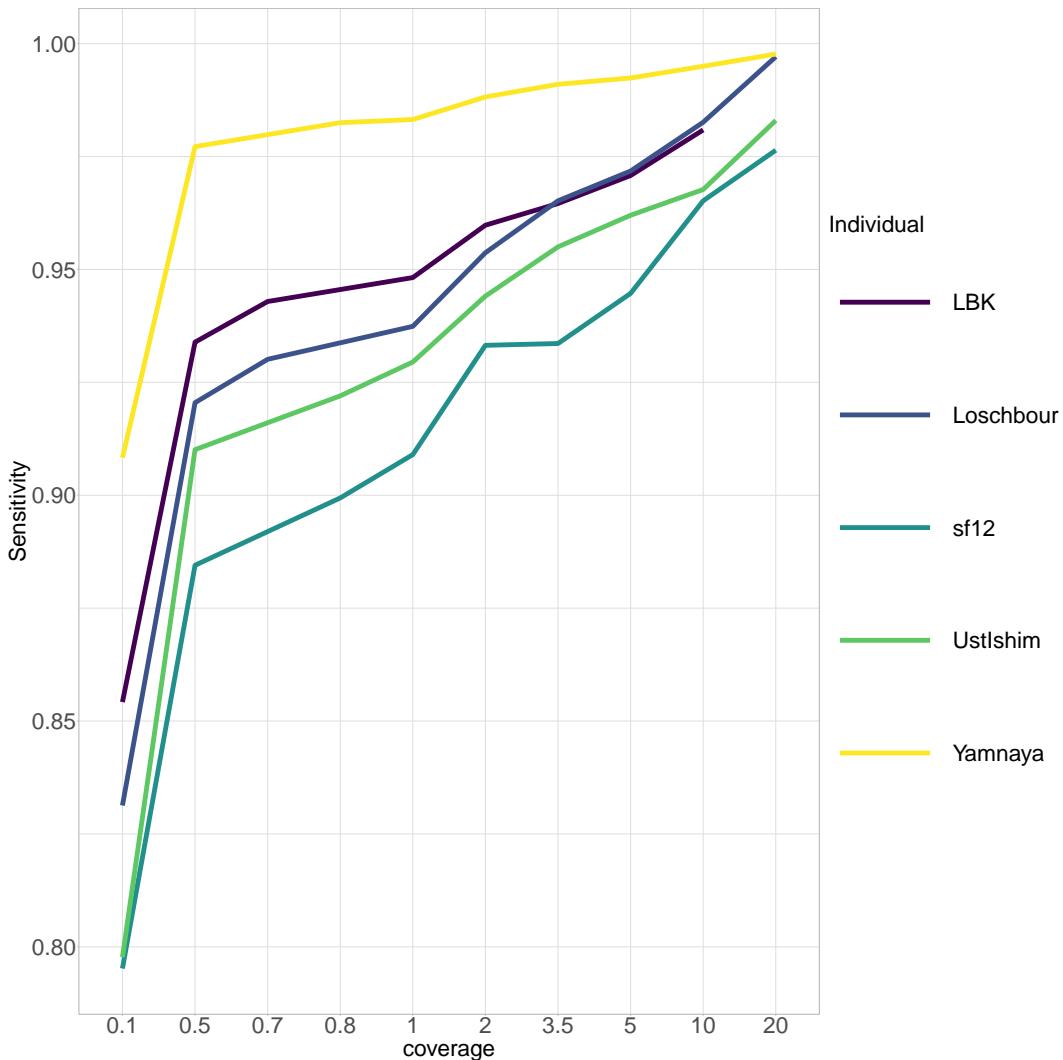


Figure 2.1: Sensitivity of genotype calling at different coverages for different ancient individuals, assuming calls in the full coverage genome are correct, calculated using rtg-tools.

accuracy.

Imputation accuracy may also be related to demographic history. Populations which are known to have smaller effective population size, such as Western-Hunter Gatherers, also contain longer tracts between individuals which are identical by descent (IBD) [46] and fewer heterozygous positions. As imputation relies on matching IBD tracts between individuals, imputation accuracy increases where individuals share more IBD [47]. Additionally, switch-errors during the pre-phasing step of imputation may harm imputation accuracy, so a

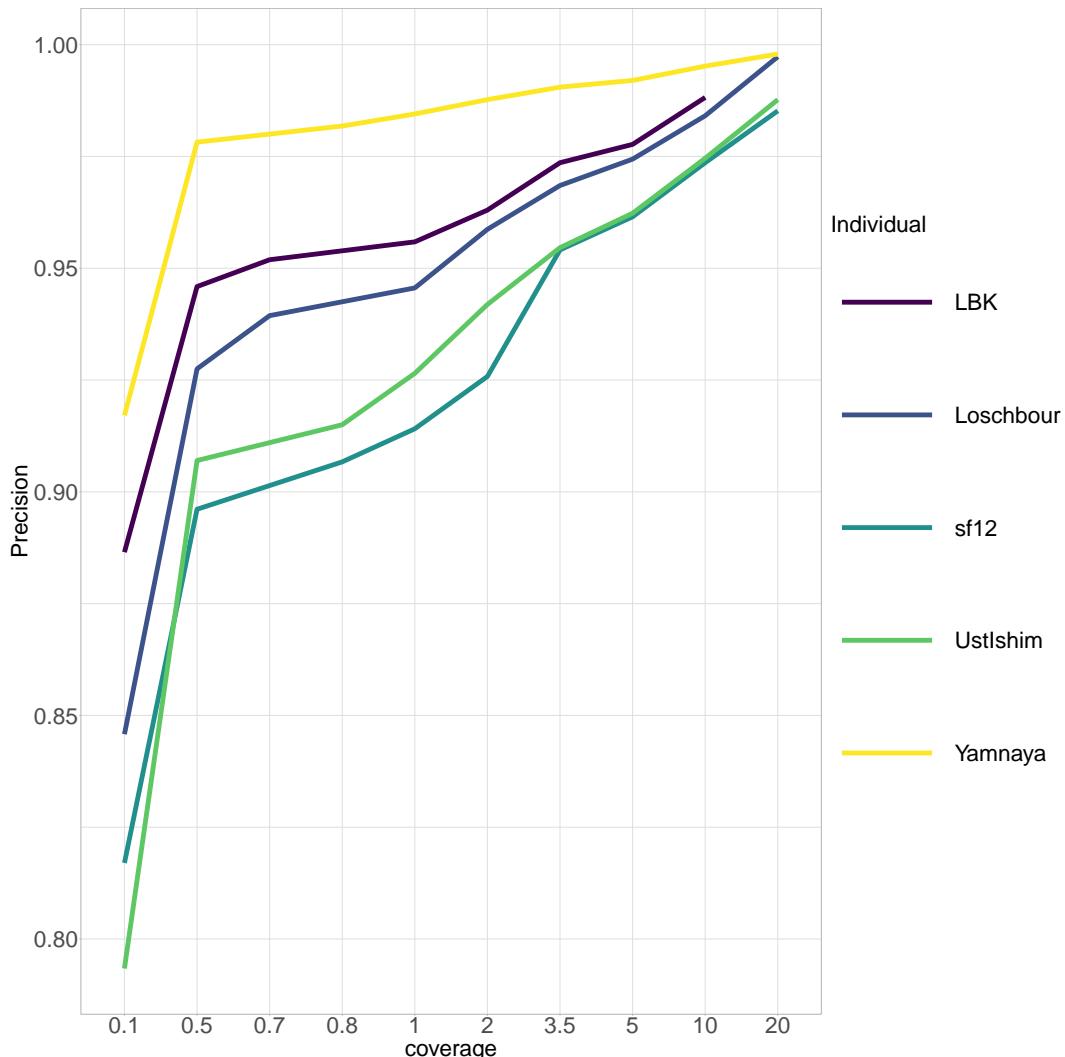


Figure 2.2: Precision of genotype calling at different coverages for different ancient individuals, assuming calls in the full coverage genome are correct, calculated using rtg-tools.

reduced density of heterozygous positions may result in increased accuracy.

2.5.2 Phasing accuracy

I also used rtg-tools to calculate the number of phased heterozygous genotypes where the downsampled individual has the same phasing as the full coverage individual (Fig 2.3). I note that this should not be considered to be the same as estimating the switch error rate, since we do not know that the phasing in the full-coverage individual is the true phase. However, this can be used

as a rough proxy for switch errors, since it is known that phasing in lower coverage individuals is likely to be less accurate than those in the high coverage individuals [24].

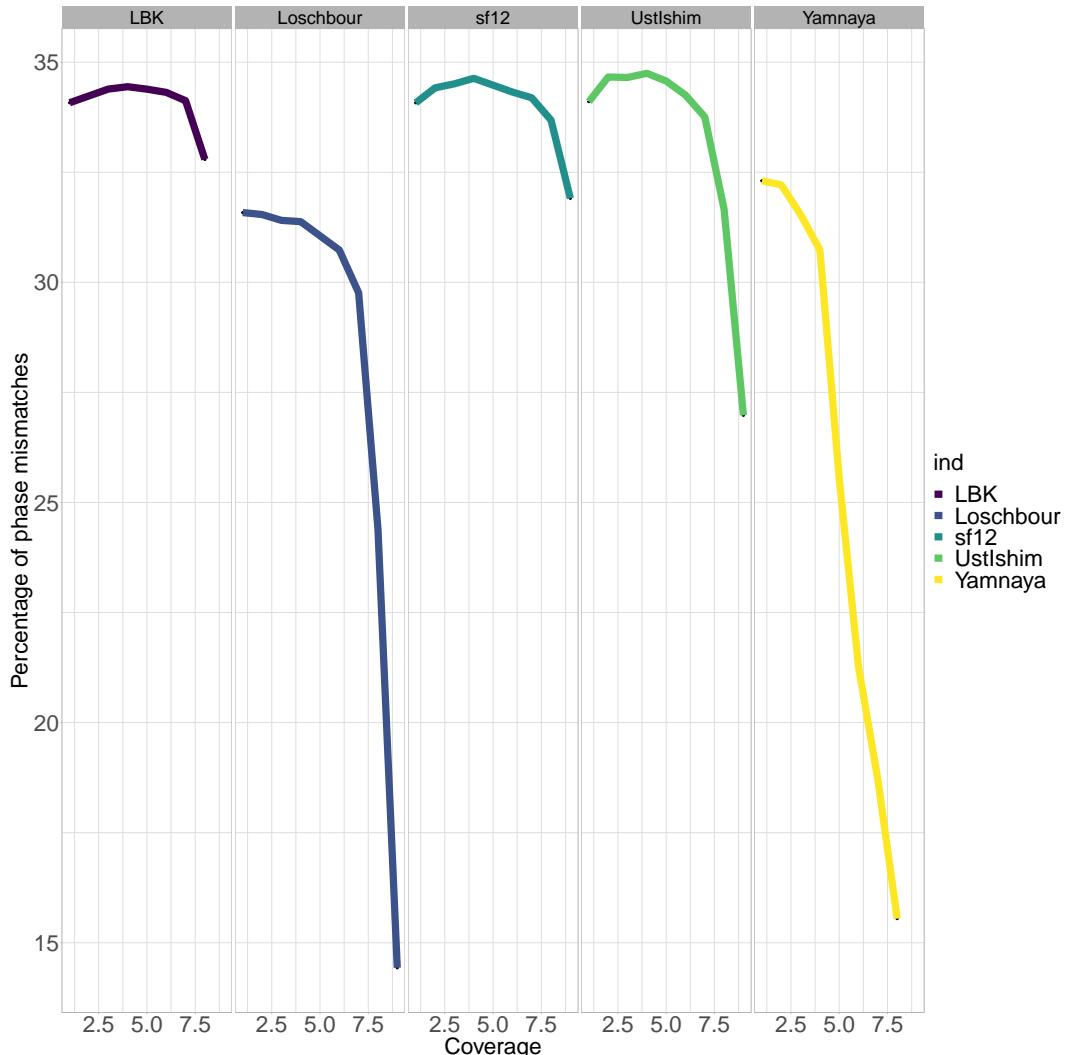


Figure 2.3: Percentage of phased genotypes which agree with the reference for each individual and each level of downsampling. Genotypes with phase deemed unresolvable by rtg-tools were excluded from the calculations. Note that these numbers are given as incorrect / (incorrect + correct - unresolved) and so values are in part driven by the relative heterozygosity of each sample.

2.5.3 Validating posterior probability calibration

GLIMPSE estimates genotype probabilities at each genotype within each individual analysed. Genotype probabilities provide information on the posterior

probability that a given genotype within a single individual is correctly called. I assessed how well-calibrated these probabilities are in the Yamnaya 0.1x downsampled individual, using the maximum genotype likelihood at each of the approximately 77 million positions which were processed by GLIMPSE. A high $\max(GL)$ for a particular genotype (i.e. 0.99) corresponds to a high confidence in the genotype. Alternatively a flat $\max(GL)$ (i.e. 0.33) corresponds to no information about the genotype.

I split the genome into 10,000 bins according to $\max(GL)$. For each bin, I calculated both the proportion of SNPs which were correctly imputed (i.e. that matched the same high coverage individual) and the mean $\max(GL)$ (Fig. 2.4). If the genotype probabilities are well calibrated, we would expect to see a clear positive linear relationship between $\max(GL)$ probability and the probability that genotype matches the full-coverage sample.

The probabilities are well calibrated ($r^2 = 0.981$) and could therefore be used for downstream analysis. It should be noted that they are slightly conservative, in that a majority of the points in Fig. 2.4 are above the line of equality. For example, the mean proportion of correct genotypes within all bins where $0.73 < \max(GL) < 0.76$ was 82%. I performed the same analysis using different samples at different levels of coverage and the results were qualitatively similar (result omitted).

2.5.4 ChromoPainter analysis

To assess the impact of coverage on ChromoPainter analysis, I merged the dataset of downsampled individuals with the ‘standard set’ of ancient reference individuals (124 ancient samples $> 2X$ coverage) and performed an ‘all-v-all’ painting of the merged dataset, which separately paints each individual as a recipient using all other individuals in the dataset as donors. The ‘all-v-all’ painting was necessary to paint the 124 ‘standard set’ of individuals against one another so that they can act as surrogates in later SOURCEFIND analysis.

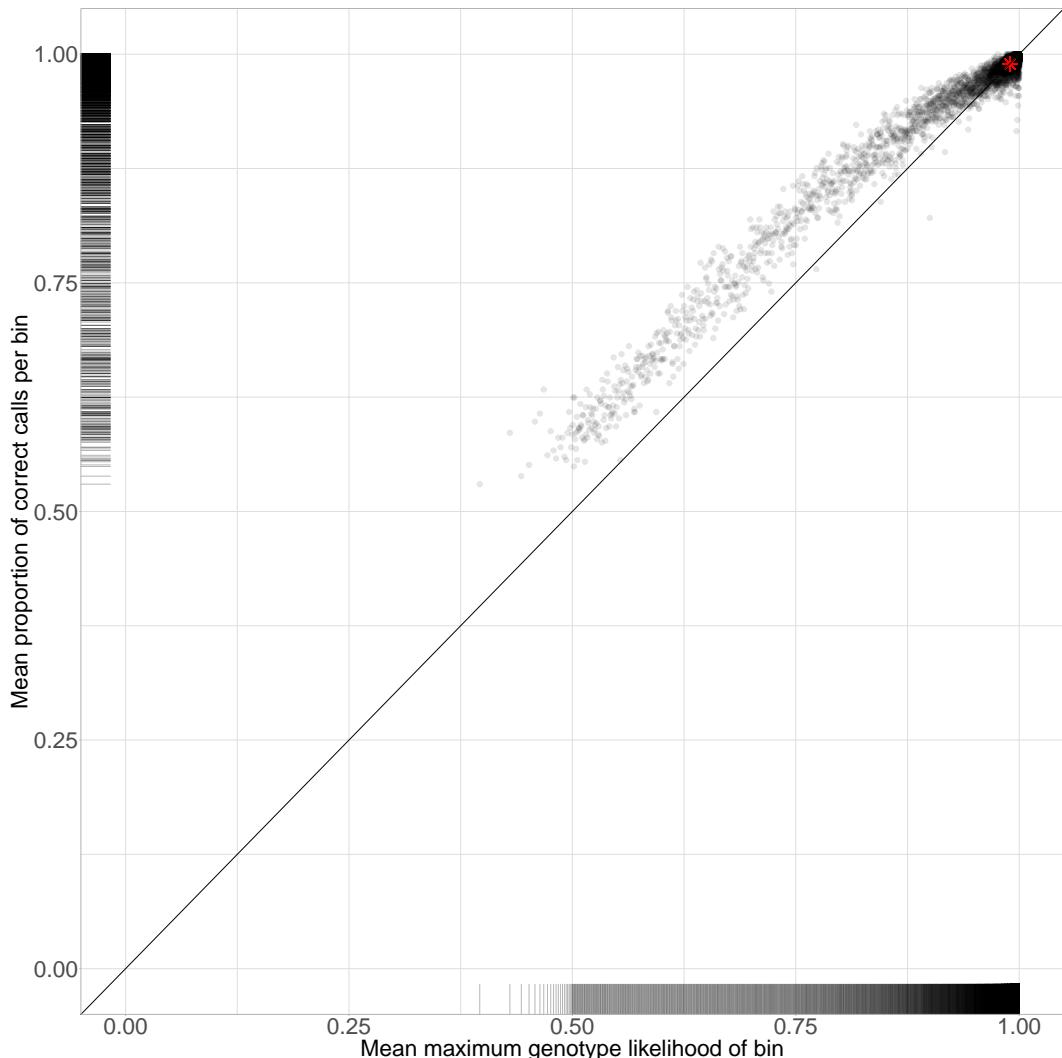


Figure 2.4: Relationship between genotype likelihood and probability of genotype call being correct for Yamnaya downsampled to 0.1x coverage. Genome binned by maximum posterior genotype likelihood and mean maximum posterior genotype likelihood (x-axis) and proportion of correct calls per bin (y-axis). Rugs on each margin show the distribution of x and y values. Black line is $y = x$.

I was interested to see whether a downsampled individual and full coverage had similar copyvectors, or in other words, whether they matched similar amounts to the same donor individuals. To do this, I estimated r-squared between the copyvectors of the full coverage and downsampled individuals.

Fig. 2.5 displays the relationship between copyvectors for each downsampled individual the corresponding full coverage individual for both 0.1x and 0.5x coverage. Each individuals' copyvectors were estimated using the same set of ancient samples as donors.

As expected, the TVD between the full-coverage and downsampled copyvectors decreased with coverage. The 0.1x genome had a substantially increased TVD, similar to the much reduced imputation accuracy. For each of the genomes downsampled to 0.1x, a particular difference to the 0.5x downsampled genomes is that the lowest contributing donors contribute more to the 0.1x downsampled genome than to the full coverage genome and that the highest contributing donors contribute less to the 0.1x genome than they do the full coverage genome. Put in other words, the copyvectors at 0.1x are tending towards becoming more ‘flat’, or copying the same amount from each donor individual.

This can also be seen as ‘regressing to the prior’. In this case, the prior is copying an equal amount to each donor individual. This can be visualised explicitly by calculating TVD between each downsampled genome and a flat prior, a vector of length D , where D is the total number of donor individuals and each element of D is equal to $1 / D$ (Fig. 2.6). This clearly shows the reduced TVD to the flat copyvector for the 0.1x individual relative to other coverages. In later sections, I will discuss whether this is ‘noise’ or ‘bias’ induced by imputation.

I also considered the effect of coverage on the copyvectors estimated when using modern individuals as donors (Fig. 2.7). I merged the downsampled and

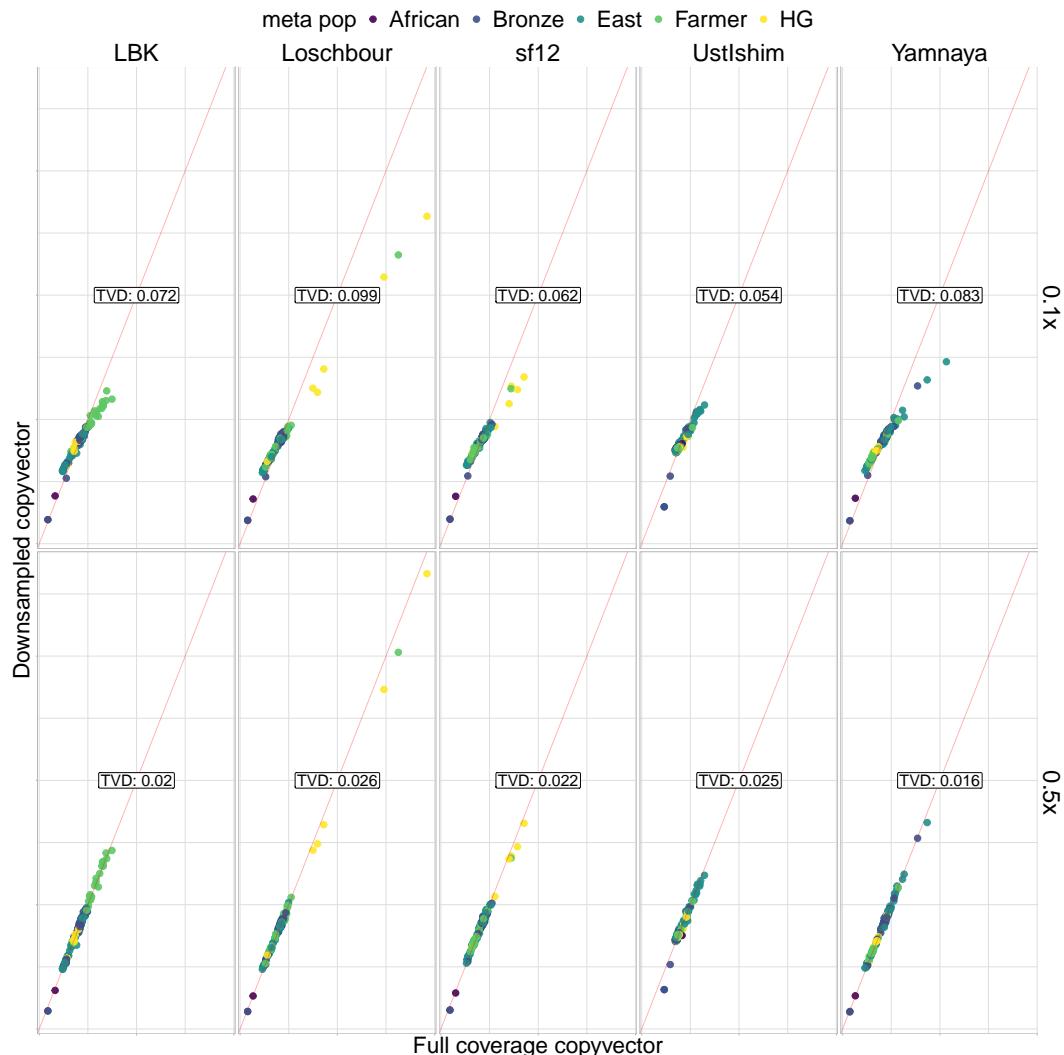


Figure 2.5: For five different samples (columns), the proportion of DNA that each downsampled (y-axis) or full coverage (x-axis) genome matches to each of 125 ancient individuals (dots). Results are shown for 0.1x (top row) and 0.5x (bottom row) downsampled genomes. Points coloured by manual assignment to broad-scale populations. Red line is line of equality ($y = x$). x and y units are normalised copying values and thus removed for clarity.

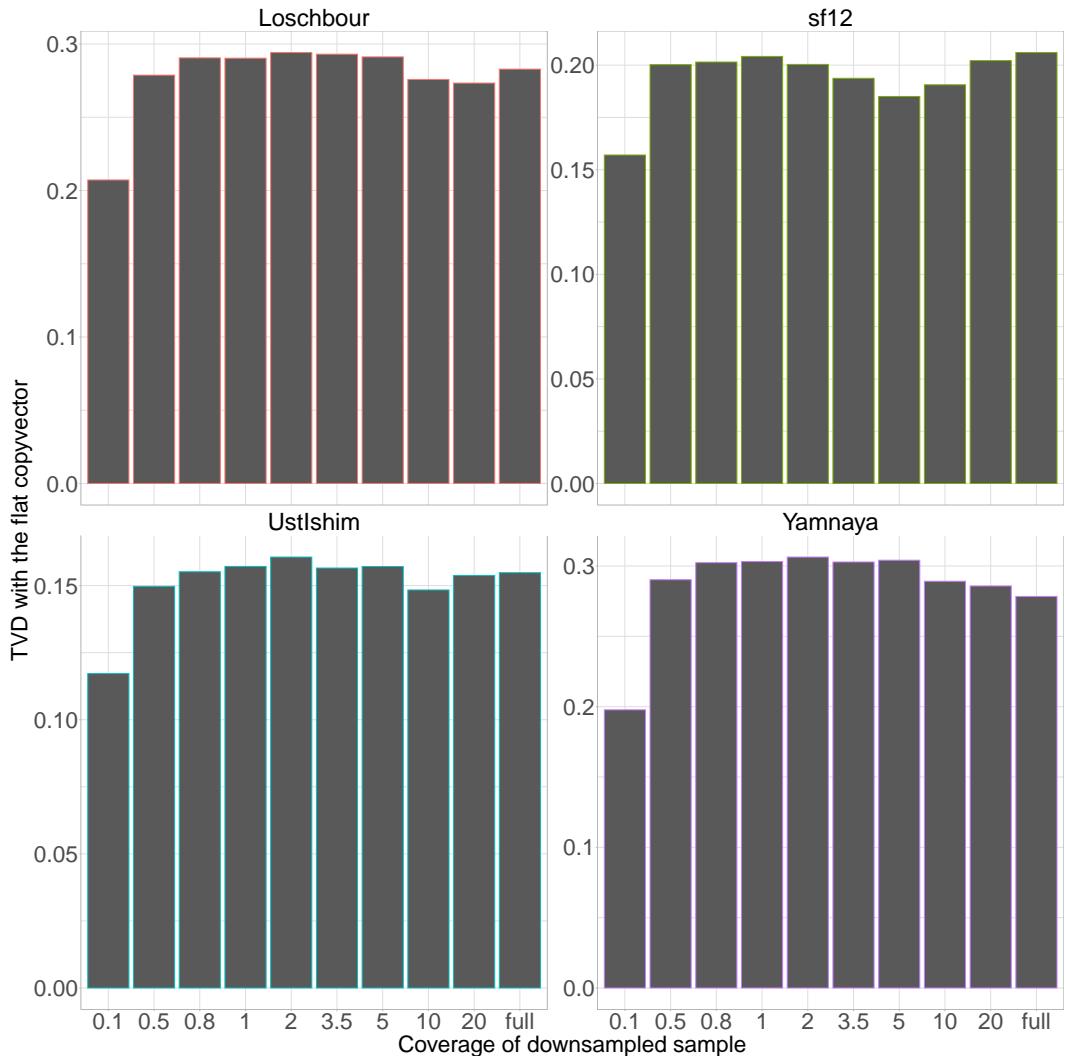


Figure 2.6: TVD (metric of copyvector dissimilarity between two individuals) between each downsampled ancient individual and a flat copyvector. Flat copyvector equivalent to a vector of length N where each element = $1/N$.

full coverage ancient individuals with the thousand genomes dataset (described in detail in appendix A.5). As was the case with the all-v-all ancients painting, the TVD between copyvectors was highest for the 0.1x individuals. However, the copyvectors show a strong correlation for 0.5x individuals.

It should be noted that utility of painting different ancient individuals with a modern reference panel depends on the ancestry and age of the ancient sample. As a comparison, I painted each full coverage and down-sampled ancient against a set donor individuals from 26 present-day populations. The spread of points along the $y = x$ line in Fig. 2.7 shows how much a particular ancient recipient preferentially copies more from particular modern population over others. LBK, for example, has points which are spread evenly across $y = x$, showing that they copy much more from some populations than others, suggesting modern populations are good for distinguishing this particular ancient sample. On the other hand, the points for Ust'Ishim are clumped together along lower values of $y = x$, showing that the copyvector is relatively flat and that it does not preferentially copy from some populations to the same degree that LBK does. This is consistent with findings that UstIshim did not contribute ancestry towards present-day populations [26]. Accordingly, relatively less useful information is obtained from painting Ust'Ishim with a modern reference panel than LBK.

Principle component analysis (PCA) is a widely used technique to visualise the relative genetic diversity of different individuals. PCA can be performed on the chunklengths matrix in a similar way to a PCA on the genotype dosage matrix, which is often employed in ancient DNA studies. Visualising whether downsampled individuals cluster close to the same sample at full-coverage is a useful way of determining whether the copyvectors of the downsampled individual reflect those of the full-coverage individual.

The position of the full coverage individuals are consistent with prior knowledge about their ancestry (Fig. 2.8). For example, Loschbour is positioned

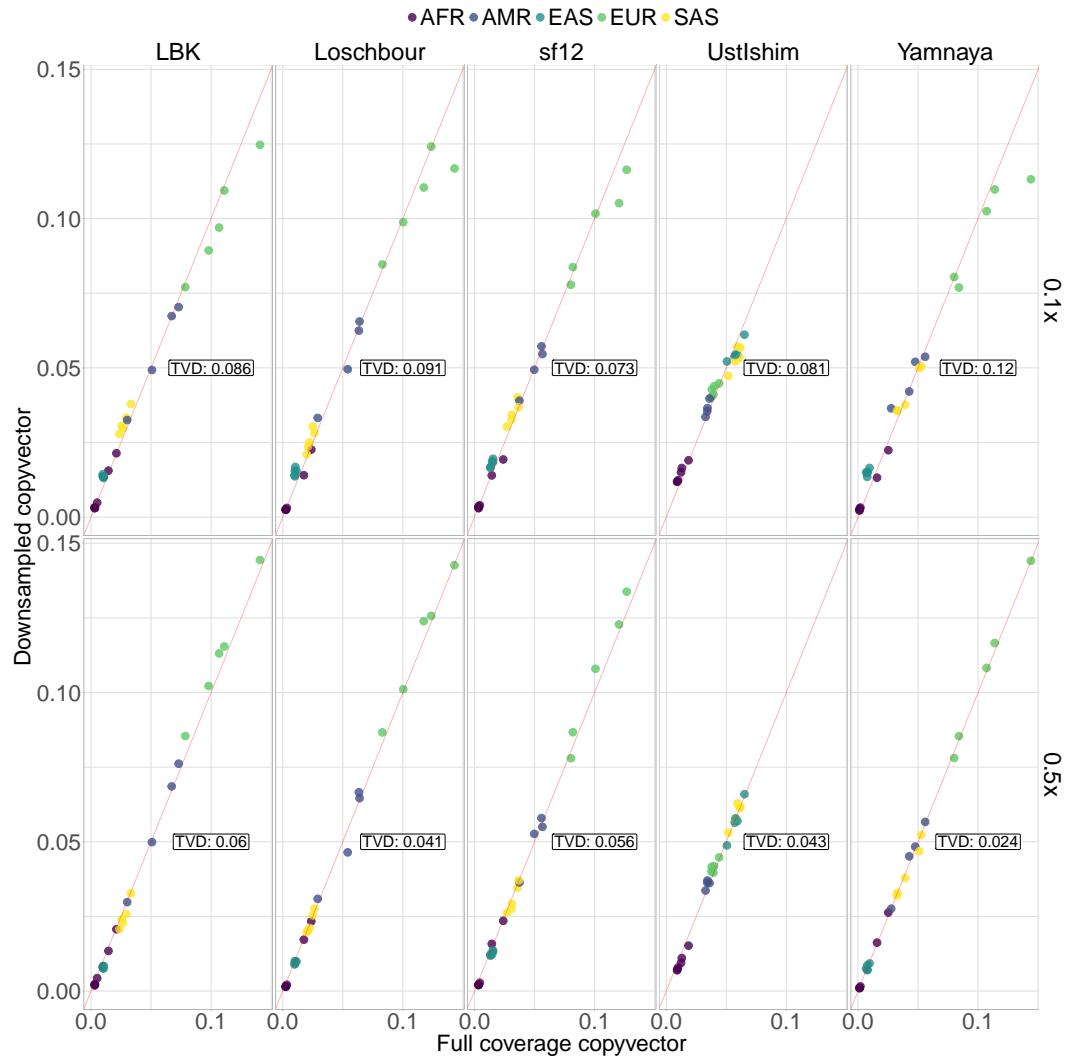


Figure 2.7: For five different samples (columns), the proportion of DNA that each downsampled (y-axis) or full coverage (x-axis) genome matches to individuals from each of 26 present-day populations (dots). Red line is $y = x$. x and y units are normalised copying values and thus removed for clarity.

alongside other Hunter Gatherers, who are highly differentiated from the later Neolithic farmers and Bronze Age Europeans. sf12 clusters with the other Scandinavian Hunter Gatherers in the dataset. Yamnaya is differentiated from the group of Bronze Age individuals and situated close to individuals from the Poltavka and Srubnaya culture. LBK is located with other individuals from the early to middle Neolithic in central Europe. Consistent with sharing little ancestry with any group over another, UstIshim is positioned close to the central Bronze Age mass, where most of the individuals in the PCA are located.

For all levels of downsampling other than the 0.1x, the downsampled and full coverage genomes were positioned very closely to one another on the PCA. When considering all downsampled individuals, a pattern emerges whereby the genome downsampled to 0.1x for each individual is ‘pulled’ towards the origin of the PCA. This may reflect a ‘homogenisation’ of low coverage genomes when many genotypes are imputed.

Taken together, these data suggest minimal effect of coverage down to and including 0.5x mean depth.

2.5.5 SOURCEFIND

I next determined the effect of sequencing coverage on the ancestry proportions estimated by SOURCEFIND. The chunklengths matrix contains information about the total length of genome one particular individual most closely matches to any other individual. However, this information is often noisy due to phenomena such as incomplete lineage sorting and variable donor group sizes. Therefore, it is often desirable to model out this noise and estimate ancestry proportions in each individual, which are cleaner and more interpretable than raw chunk lengths.

I began by considering three ancestral sources, or ‘surrogates’, fixed as

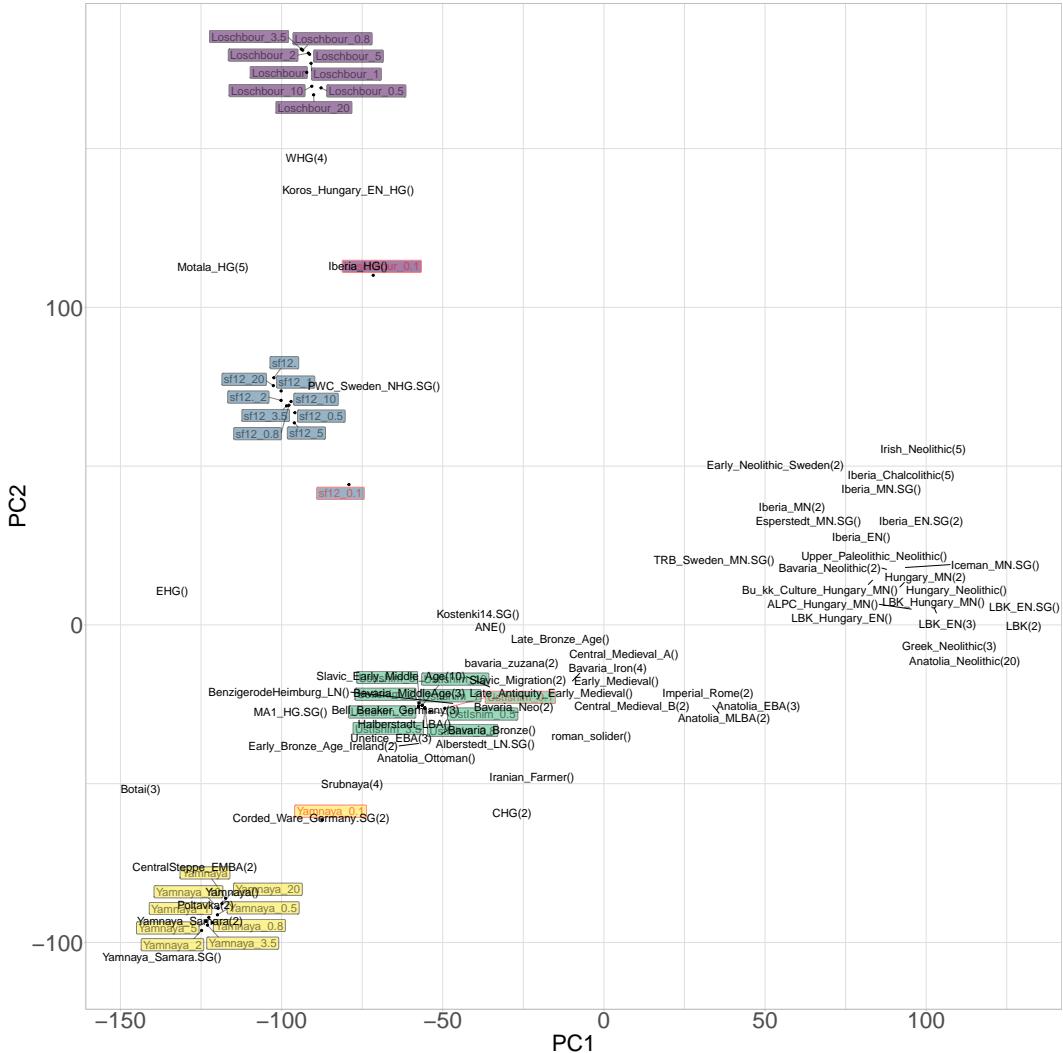


Figure 2.8: Principle component analysis (PCA) of downsampled, full coverage and downloaded ancient individuals generated from the linked chunklengths matrix. Full coverage and downsampled genomes of the same individual are coloured the same. Reference individuals are grouped into populations plotted as the mean principle components for all individuals within the population. Numbers in labels correspond to the number of individuals within the reference population. 0.1x samples have red border for clarity.

Anatolia Neolithic, Western Hunter-Gatherer and Yamnaya steppe pastoralist. I compared inferred proportions for the same individual across different levels of coverage (Fig. 2.9).

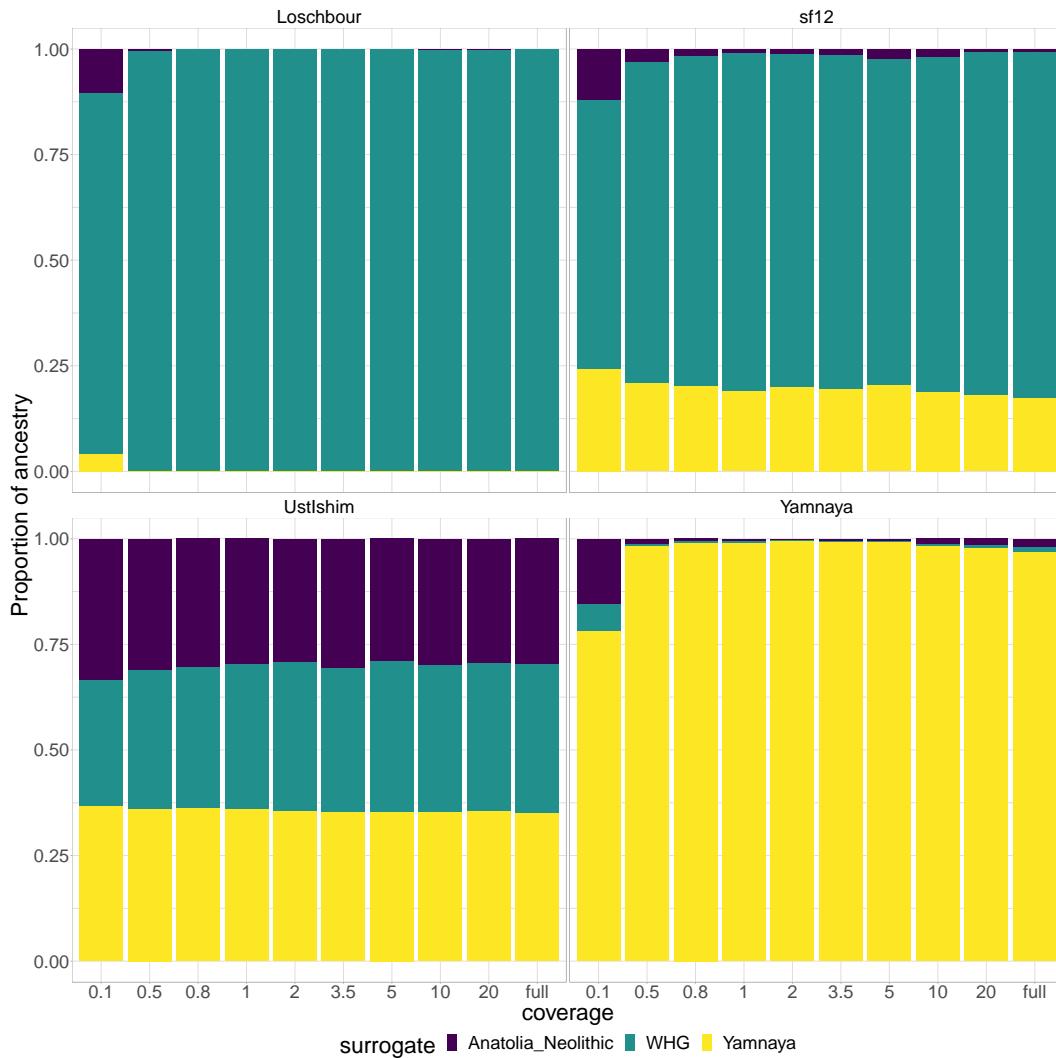


Figure 2.9: Each panel gives inferred recent ancestry sharing proportions for a different downsampled genome. Bars represent proportion of ancestry, coloured by different surrogates. Different coverages for the same individual are given within each panel. Three surrogates were used.

The results suggest that SOURCEFIND estimates are robust down to 0.5–0.8x coverage. At 0.1x coverage, there is an increase in ancestry components that are not present in higher coverage samples, suggesting they are artifacts caused by low coverage. For example, small components of Anatolia Neolithic and Yamnaya ancestry appear in Loschbour at 0.1x coverage, which are not

present at any higher coverages. Above 0.5x coverage, the effect of coverage on estimated ancestry proportions appears to be marginal. For example, in sf12, the difference in the minor ancestry component of Anatolia Neolithic is, at most, 2.369%.

However, more than three surrogates are often used, as SOURCEFIND is meant to infer the most important contributors without a priori knowledge of the samples' ancestry. Therefore, I re-ran SOURCEFIND using 39 surrogate populations.

Again, Loschbour seems to be the least affected by coverage, with only slight differences between the 0.5x and full coverage samples. It is known that Upper Paleolithic / Early Neolithic Hunter-Gatherer populations were small and lacked genetic diversity [16, 48, 49]. It is therefore expected that Hunter-Gatherers would share longer IBD segments than individuals from outbred populations. Accordingly, this may make estimating SOURCEFIND proportions easier.

2.6 Issues and possible solutions for low coverage ancient DNA

The previous section outlined a drawback of performing ChromoPainter analysis on low coverage (less than 0.5x) ancient DNA samples; low coverage samples appear to be shifted towards the origin of a principle component analysis (PCA) relative to the same sample at higher coverage (Fig. 2.8). This is evident for the lowest coverage samples at 0.1x and suggests that samples of this coverage cannot be reliably analysed using current methodology.

In order to solve the issue of coverage-related bias, it is first necessary to determine at which stage of the analysis pipeline this bias is introduced. By 'analysis pipeline', I refer to the stages of variant calling, imputation and

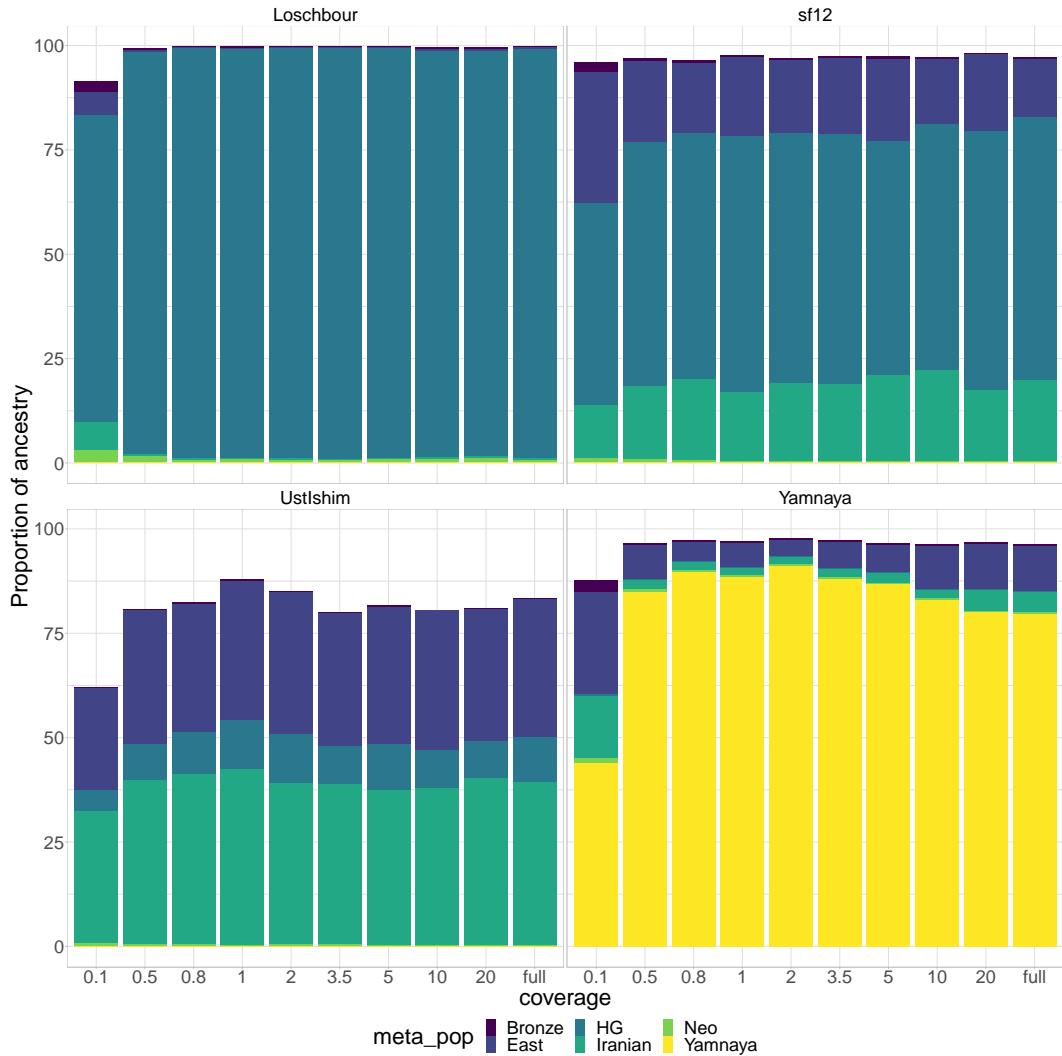


Figure 2.10: Each panel gives information for a different downsampled genome. Bars represent proportion of ancestry, coloured by different surrogates. Different coverages for the same individual are given within each panel. All 39 ancient surrogates were used. Only surrogates with more than 5% are shown. Surrogate populations coloured by

phasing, and ChromoPainter described in the methods section.

2.6.1 PCA imputation test

To explicitly test at what stage the bias is introduced, I performed a set of principle component analyses on the downsampled data. First, I performed PCA projections of all downsampled ancient individuals onto a set of present-day European individuals using i) pre-GLIMPSE genotypes and ii) post-GLIMPSE (imputed) genotypes (Fig. 2.11). PCA projections are used when the target dataset, in this case downsampled ancients, contain variable levels of missing data.

The results show that there is no apparent coverage-related bias in the pre-GLIMPSE PCA; the 0.1x samples do not substantially differ in their position from the other downsamples of the same individual. However, there is a degree of noise; for example, the LBK downsamples are spread over a small region on the PCA.

On the other hand, the 0.1x samples are clearly shifted to the centre of the post-GLIMPSE PCA, away from the full coverage individual and other downsamples. This suggests that coverage-related bias is being introduced in the imputation stage. GLIMPSE appears to have removed some of the noise in the downsampled individuals. For instance, the noise observed in the LBK samples in the pre-imputation PCA is substantially reduced and the samples cluster more tightly.

I also performed a PCA, using the same set of present-day European samples and downsampled ancient individuals as previously, but on the chunklengths matrix ChromoPainter output. There is an increased amount of noise and evidence of coverage-related bias relative to the post-GLIMPSE genotype PCA. Fig. 2.11) displays the PCA for the same painting, but using the unlinked chunkcounts matrix. Comparing the linked and unlinked PCAs shows the effect

of including linkage (i.e. haplotype information) on the amount of bias and noise across each sample. Per-sample, there is reduced noise in the unlinked painting.

These results suggest that imputation introduces a degree of bias into 0.1x samples that is not apparent on non-imputed genotypes. They also suggest that ChromoPainter introduces an additional degree of bias, or that it amplifies bias already present introduced at the imputation stage. Accordingly, removing SNPs which have been poorly imputed may be a way to mitigate such biases.

2.6.2 Direct imputation test

The previous section suggested that imputation plays a role in the introduction of coverage-related bias. However, it is not clear whether it is ‘bias’, i.e. towards the reference population used to assist imputation, or ‘noise’ due to random incorrect imputation. To directly test whether the effect of imputation is noise or bias, I used the Human Origins dataset (described in appendix A.19), containing the genotypes of 5998 present-day individuals from across the world, genotyped at 560,442 SNPs. I chose to use present-day samples because there is a larger total number of individuals and larger number of individuals per population, giving more power to detect any potential bias. Additionally, the populations in present-day samples are more homogenous and well-defined compared to ancient groups. I set all but 70,000 SNPs as missing and imputed missing positions using the HRC as a reference, in order to simulate a dataset where the majority of SNPs are imputed. I then performed an all-v-all painting of i) the original Human Origins dataset where none of the 560,442 SNPs had been imputed and ii) the simulated dataset where 430,000 SNPs had been imputed.

Bias occurs when missing genotypes are incorrectly imputed with variants from certain populations more frequently than others. We might expect these populations to be those which are more prevalent in the reference panel, or

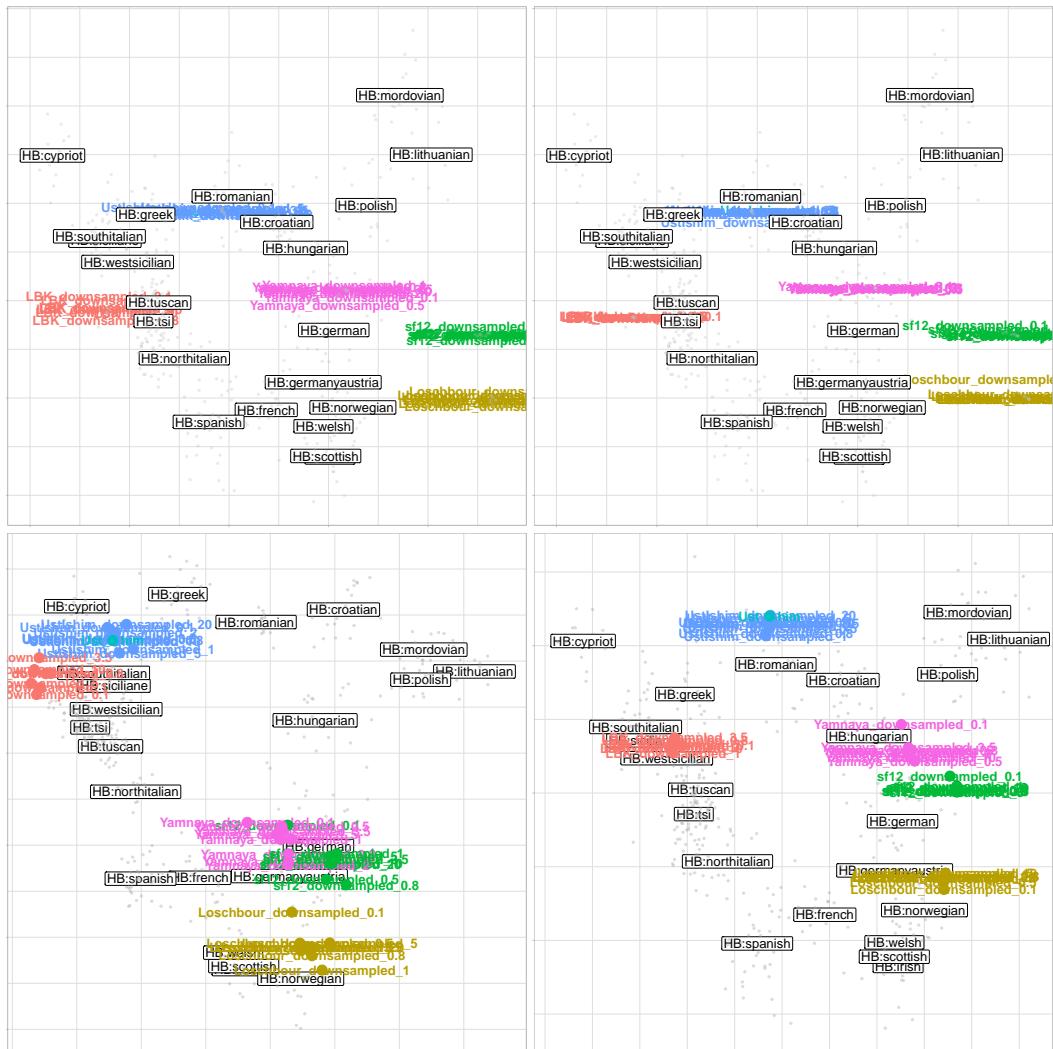


Figure 2.11: Principle Component Analysis. Top Left - pre-GLIMPSE genotypes. Top Right - post-GLIMPSE genotypes. Bottom Left - ChromoPainter Linked. Bottom-Right - ChromoPainter Unlinked. White labels correspond to the midpoint of all samples from that population, grey points correspond to modern individuals.

from populations closest to the target population. We would correspondingly expect bias to mean that, when painted, some donor populations would donate more than others, relative to if no imputation had taken place. On the other hand, if ‘noise’ is dominating results, we would expect the incorrectly imputed genotypes to be randomly distributed across populations and similarly, we would not expect to see any populations donating more than others relative to if no imputation had taken place.

Therefore, we can compare the amount different donor groups donate under the imputed and non-imputed SNP set by plotting the mean amount donated by each population using imputed SNPs and non-imputed SNPs (Fig. 2.12). The 20 populations that contribute most are either European or Jewish. Notably, the Haplotype Reference Consortium panel which was used to imputed the data consists primarily of individuals of European descent. The two populations which are over-copied the most after imputation are two English populations from Kent and Cornwall. This suggests that there is a clear bias towards copying more from European populations when the data has been imputed using the HRC.

2.7 Solutions

In this section I will explore potential solutions to the issue of coverage-related bias. Based on the findings in previous sections, imputation causes bias towards particular reference populations in modern samples.

2.7.1 Accounting for allele likelihoods

Section 2.2.1 describes an improvement to the ChromoPainter algorithm. Instead of assuming that each allele on a haplotype is correct with a probability $1 - \theta$, where θ represents an error probability, the posterior genotype probability from GLIMPSE is accounted for in the emission probabilities of the copying

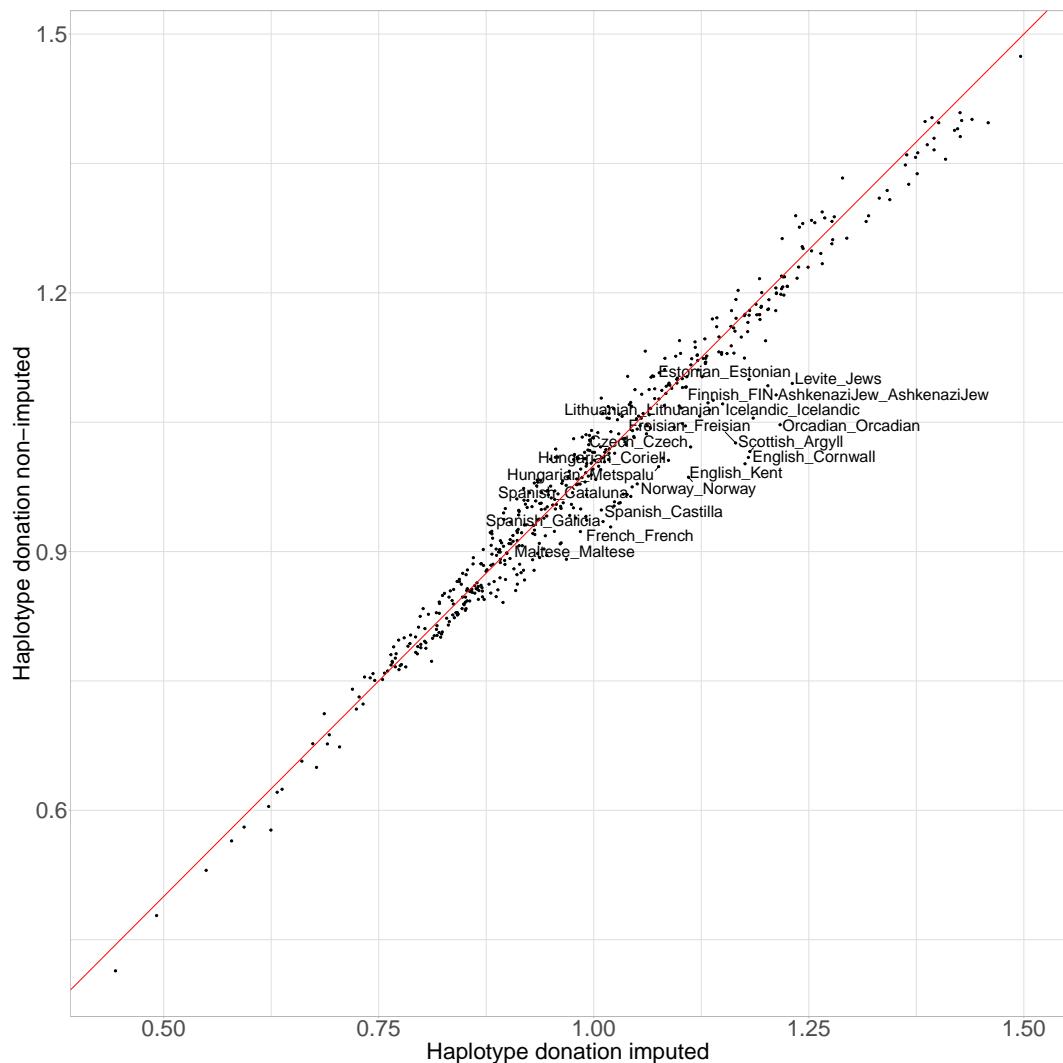


Figure 2.12: Comparison of the mean normalised cM donated by each donor population using the imputed and non-imputed SNP sets. The 20 populations with the largest different between imputed and non-imputed donation are highlighted.

model. The motivation behind this update is that the uncertainty associated with genotype calls at low coverage is suitably propagated throughout the painting process, resulting in uncertain alleles contributing less towards the expected copying values than more certain ones. This is similar in spirit to that of Viera et al (2016), who account for genotype likelihoods to infer inbreeding IBD tracts from low coverage sequencing data [50].

To determine whether accounting for allele likelihoods improved the painting accuracy of a low-coverage genome, I painted the individuals downsampled to 0.1x and 0.5x and corresponding full coverage samples using the ‘standard set’ of ancient reference individuals, using both ChromoPainterV2 and ChromoPainterV2Uncertainty. I then calculated r-squared between the copyvectors of full coverage and downsampled individuals using the two different methods (Fig. 2.13). This shows that at 0.1x, the ChromoPainterV2 method clearly outperforms ChromoPainterV2Uncertainty across all samples, whereas at 0.5x, the new method marginally outperforms the standard method. Therefore, while accounting for allele likelihoods may improve performance in cases of coverage $\geq 0.5x$, which has been shown to still capture some haplotype information, it does not help in cases of coverage of 0.1x where bias problems persist.

2.7.2 Filtering SNPs

In this section, I will test whether filtering the set of input SNPs on different criteria reduces the effect of coverage related bias.

The frequency of a particular variant in the reference panel (RAF - reference allele frequency) used for imputation is known to affect how accurately that variant can be imputed [18, 24, 31, 51]. Specifically, we expect variants which are less frequent in the reference panel to be imputed at a lower accuracy than those which are more frequent. Therefore, removing variants with a low frequency in the reference panel may mitigate the coverage related bias by removing variants which have been incorrectly imputed. In other words, we

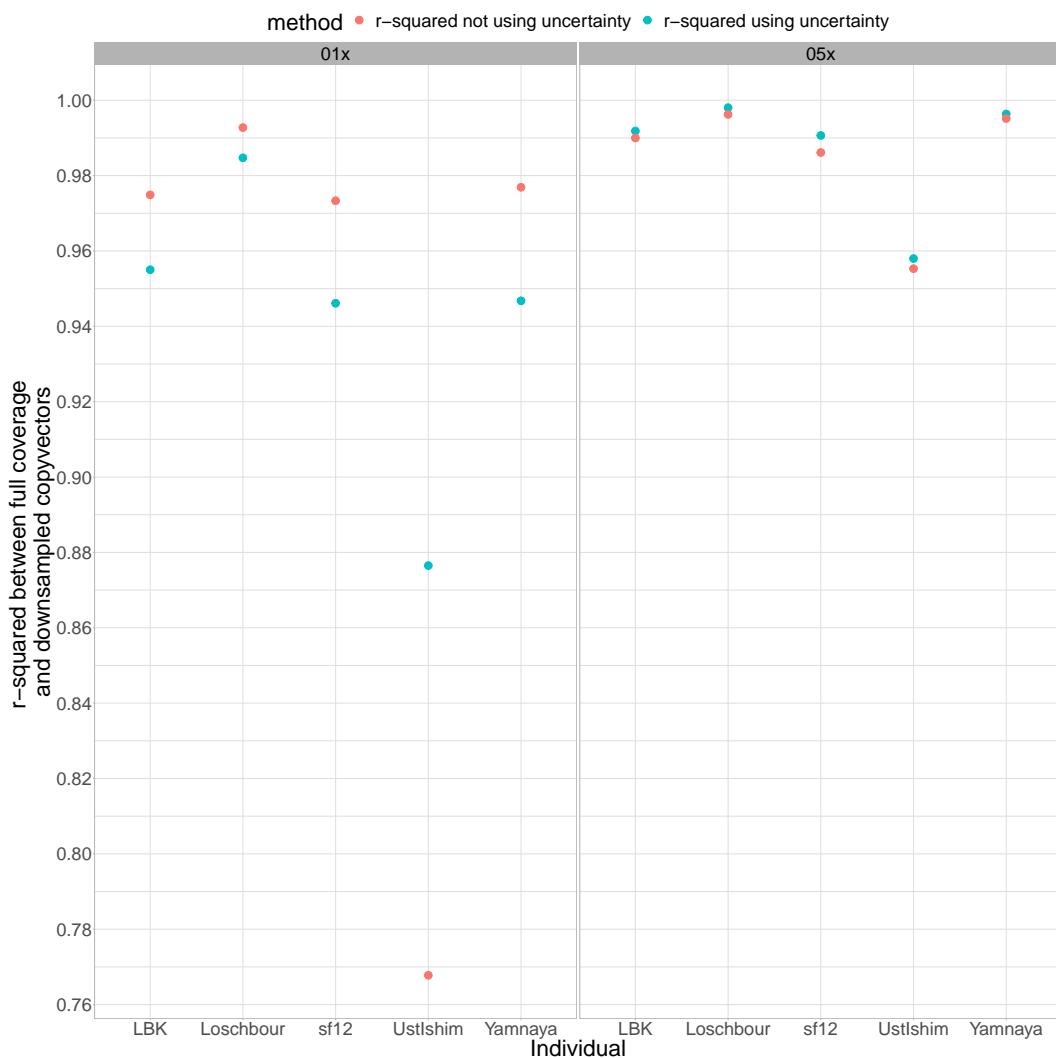


Figure 2.13: Comparison of performance of ChromoPainterV2 and ChromoPainterV2Uncertainty. Panels correspond to samples downsampled to 0.1x (left) and 0.5x (right). Points correspond to the r-squared between the downsampled individual and the same individual at full coverage. Red points are values obtained from ChromoPainterV2 and blue points are those obtained from ChromoPainterV2Uncertainty.

sample	u_01x	u_05x	s_01x	s_05x	r_01x	r_05x	gp_05x
LBK	0.989	0.996	0.989	0.996	0.979	0.997	0.992
Loschbour	0.998	0.999	0.998	0.999	0.992	0.999	0.994
sf12	0.989	0.995	0.989	0.995	0.974	0.995	0.982
Yamnaya	0.990	0.999	0.990	0.999	0.972	0.998	0.995
UstIshim	0.848	0.992	0.848	0.992	0.930	0.979	0.969

Table 2.1: Table of r-squared values between the copyvectors of full coverage and downsampled individuals. ‘u’ refers to ChromoPainterUncertainty, ‘s’ refers to ChromoPainterV2, ‘r’ refers to filtering SNPs with reference allele frequency (RAF) $0.1 > RAF$ or $RAF > 0.9$ and ‘gp’ refers to filtering $\max(GP) \geq 0.990$.

want to retain the SNPs where both alleles are relatively common within the population.

For each individual, I took the 428,425 SNPs in the HellBus set and removed SNPs with $0.1 > RAF$ or $RAF > 0.9$, removing an average of 50,187 SNPs per individual. RAF refers to the frequency of the allele in the 1000 genomes reference panel used to phase and impute the HellBus I then painted individuals downsampled to 0.1x and 0.5x using the standard set of 125 ancient donor individuals.

Comparing the r-squared values between the copvectors showed that this did not improve the 0.5x copyvectors (Table 1.1).

I then chose to filter SNPs based on $\max(GP)$ at each position. $\max(GP)$ correspond to the accuracy with which a SNP has been imputed, with higher values reflecting a higher chance of that genotype being imputed correctly. For each individual downsampled to 0.5x, I only retained positions where the $\max(GP) \geq 0.990$. This resulted in a total of 348,852 SNPs for LBK, 339,949 for Loschbour, 315,075 for sf12, 308,961 for UstIshim and 386,484 for Yamnaya. Because different SNPs were removed from different individuals, each individual was painted separately. The same standard set of 124 ancient donors was used. Again, this did not improve the accuracy of the copyvectors.

2.7.3 Restricting analysis to non-imputed SNPs

Section 1.6.1 showed that imputation was the likely cause of coverage related bias. Thus, restricting ChromoPainter analysis to non-imputed SNPs above a certain coverage may mitigate such bias.

However, removing SNPs from the analysis may have side-effects. Linkage information increases when the genetic distance between SNPs is reduced. Similarly, increasing the genetic distance between SNPs reduces linkage information and therefore may reduce the overall power of haplotype-based methods distinguish between closely related haplotypes. At the most extreme case, retaining only a small number of SNPs may effectively reduce the method to unlinked and lose the advantage given by haplotype-based methods. This may be important if we decide to restrict analysis to non-imputed SNPs, as low coverage samples may only have a small number of high enough coverage, non-imputed SNPs. Therefore, it is important to determine whether samples of a particular coverage have enough regions containing enough high-coverage SNPs to retain the advantages of haplotype-based methods over unlinked ones.

One case study to test whether a set of SNPs has enough linkage information is to determine whether it is possible to distinguish individuals born in Devon from those born in Cornwall. This has previously been achieved using the fineSTRUCTURE clustering algorithm (linked), but not using unlinked methods (ADMIXTURE [41]) [42]. Therefore, determining whether it is possible to distinguish between individuals from Devon and Cornwall acts as a test case for reducing SNPs; do low coverage samples have enough SNPs of enough coverage to distinguish between these two populations?

To do this, I first needed to determine the average size of chunks copied in individuals from Devon and Cornwall when painted by other all POBI populations and the average number of SNPs within each chunk. I could then search the genomes of ancient individuals to determine whether they have

enough chunks with enough SNPs with an adequate level of coverage.

I first painted individuals from Devon (n=73) and Cornwall (n=89) with all other POBI individuals as donors (n=2039), using the full set of SNPs (n=452,592) and a reduced set of SNPs, retaining 12 levels of reduction between 0.2% - 90% of the original number of SNPs (a full list of the reduction levels and details of the painting procedure can be found in the methods section). Painting using a reduced set of SNPs is intended to simulate an ancient genome where there only a subset of the total number of SNPs have been covered by at least one read.

Using the painting containing the full set of SNPs, I estimated the median length of haplotypes donated from POBI counties to individuals in Devon and Cornwall. This yielded a value of approximately 500Kb. This serves as an estimate for the size of regions of good coverage SNPs in ancient individuals needed to be able to distinguish between two closely related populations. I also estimated, for each level of reducing SNPs and window size, how many ‘good’ SNPs are needed within each window. 500Kb corresponds to 6,000 windows genome-wide, and for example, 20% density is approximately 100,000 SNPs; so $100000/6000 = 16.7$ good SNPs are required per window.

Next, I wanted to determine, for each of the levels of SNP reduction, the minimum number of 500Kb windows needed to match the power of the full set of SNPs. In this context, ‘power’ means the ability to correctly assign individuals to either Devon or Cornwall based on which they have the lowest TVD with. To do this, for each level of SNP reduction, I painted each individual from Devon and Cornwall using all other POBI individuals. I then used TVD to assign each recipient individual from Devon or Cornwall in turn to either Devon or Cornwall. Specifically, I started with chromosome 22 and for each individual, calculated the TVD with the mean copyvectors of all other recipients from Devon and Cornwall, taking the minimum as the assigned population. I then added in chromosome 21 to chromosome 22, and assigned the individuals again.

n_snps	250Kb	500Kb	1Mb
40,000	7691	3879	1967
45,000	6272	3166	1607
50,000	5659	2858	1452
100,000	3602	1820	925
150,000	4083	2064	1049
200,000	4083	2064	1049
250,000	4083	2064	1049
300,000	5659	2858	1452
350,000	4507	2278	1158
400,000	4083	2064	1049
450,000	4083	2064	1049

Table 2.2: Number of 250Kb, 500Kb or 1Mb windows required at different levels of SNP reduction to match the TVD assignment power of 500K fully genotyped SNPs for individuals in Devon and Cornwall.

I continued to add chromosomes until the power to assign individuals was the same as when using the full set of 500,000 SNPs (i.e. the same proportion of individuals could be correctly assigned). Accordingly, for each level of reducing SNPs, I had the chromosomes required to match the power in the full set of SNPs. I converted this value to the total number of non-overlapping 250Kb, 500Kb or 1Mb windows (Table ??).

I repeated an identical analysis, including reducing the total number of SNPs, using individuals from the Mandenka and Yoruba ethnic groups rather than Devon and Cornwall.

Once I knew the minimum number of windows needed at each level of SNP reduction, I searched the genome of each ($n=587$, median coverage=1.1x) ancient individual for non-overlapping windows of sizes 250Kb, 500Kb or 1Mb for Y SNPs above Z coverage, varying both Y and Z .

Fig 2.14 shows the mean number of windows per individual with at least Y SNPs above Z coverage, with individuals being grouped into bins based on their mean coverage. Points are coloured yellow if, within the bin of coverage, samples have at least 2000 windows. Samples less than 0.5x do not have enough

n_snps	250Kb	500Kb	1Mb
30,000	6272	3166	1607
35,000	3099	1565	796
40,000	3099	1565	796
45,000	2612	1321	673
50,000	3099	1565	796
100,000	1886	956	489
150,000	1304	661	338
200,000	506	255	130
250,000	267	135	69
300,000	506	255	130
350,000	506	255	130
400,000	506	255	130
450,000	267	135	69

Table 2.3: Number of 250Kb, 500Kb or 1Mb windows required at different levels of SNP reduction to match the TVD assignment power of 500K fully genotyped SNPs for individuals from Mandenka and Yoruba ethnic groups.

windows if the threshold for a ‘good’ SNPs is being covered by a single read. As it is not possible to call a heterozygous position with only a single read, this suggests that there are not enough non-imputed SNPs with enough coverage to match the power seen in full coverage individuals. Indeed, even when there are 3 reads covering a site, there is still a 25% chance of not identifying a heterozygous position. Only the samples in the 2-5x coverage bin had enough windows when using a coverage threshold of 4 and 5 reads.

This analysis therefore suggests that there are not enough regions with enough high quality SNPs at mean coverages less than 2x to reliably analyse using ChromoPainter.

2.8 Discussion

Many of the analyses performed in this section only used a single target sample, as I did not identify a way to generate multiple downsampled individuals from the same population. For example, the SOURCEFIND analysis I performed

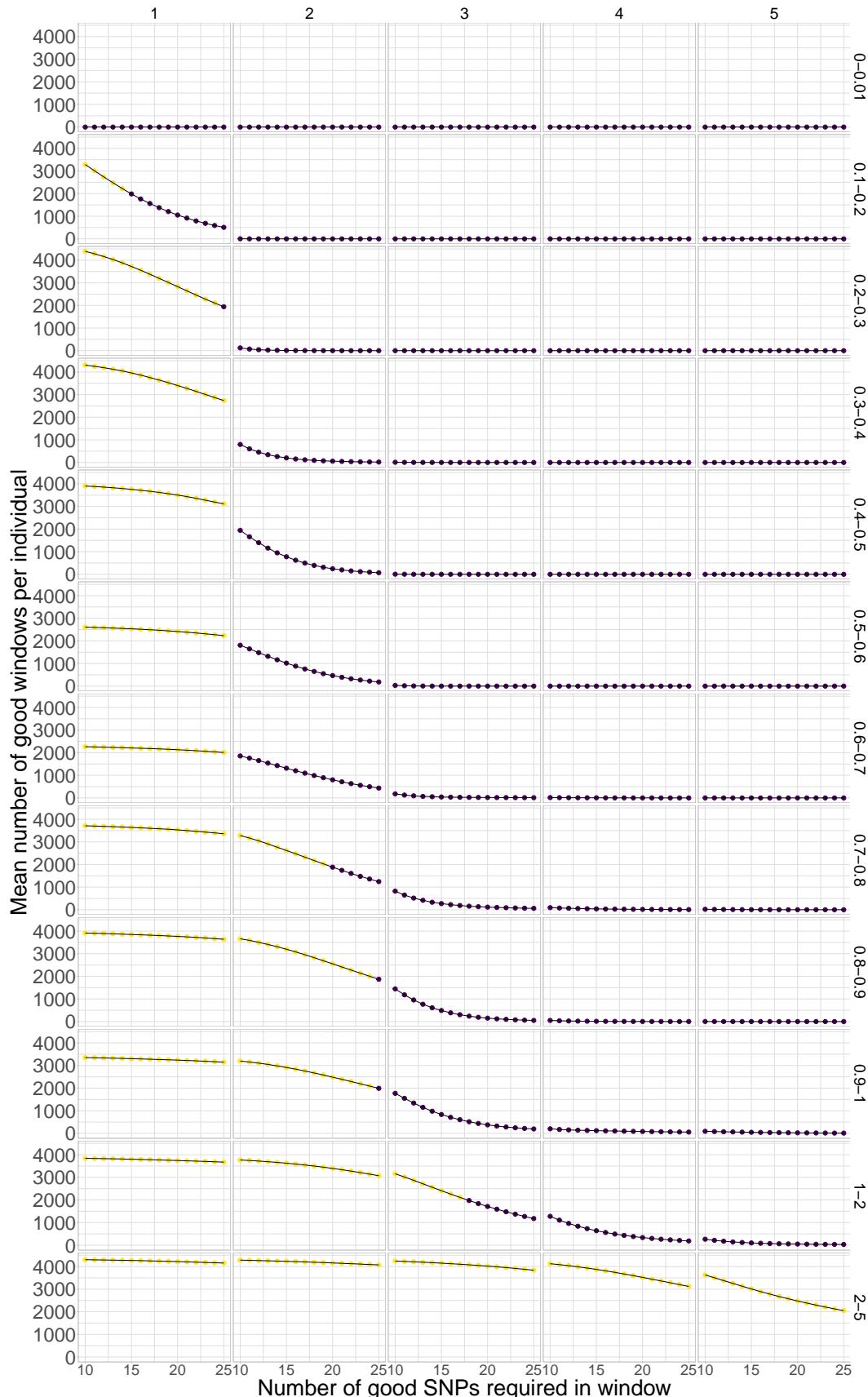


Figure 2.14: Mean number of windows (y-axis) within the genome of each ancient individuals within a given range of coverages (rows) with at least Y SNPs (x-axis) above a particular coverage Z (columns).

used a single target downsample when estimating ancestry proportions. This differs from a typical ancient DNA analysis, such as those of Margaryan et al [20], where there may be up to 20 low coverage samples per population. This number may increase in the future as the technology to generate ancient DNA improves. Leveraging information across multiple samples from the same population would improve the accuracy of population-wide ancestry or admixture estimates, for example. Thus, the results presented in this section which used a single target individual may underestimate the ability to analyse low-coverage samples. It may be possible to accurately analyse 0.1x samples if there are multiple samples per population.

In this section I used present-day individuals to estimate the number and size of chunks needed to retain haplotype information. This was because present-day individuals are simpler to analyse; the populations are better defined than in ancient samples (i.e. it is possible to only include individuals whose grandparents were born within 100kM of a target location), are of uniform coverage and contain many more individuals per population. Thus, using present-day individuals removes potentially confounding factors that may be present when analysing ancient samples. However, using present-day samples to draw conclusions about ancient samples may lead to underestimating the number of SNPs per window required. As the present-day samples had been genotyped high-quality DNA samples and a genotyping array, each genotype can be called with a high confidence. This is not the case with ancient samples, where each SNP may be covered by a small number (<3) of reads.

Chapter 3

Investigating the sub-continental ancestry of ethnic minorities within the U.K. Biobank from sparse genotype data

3.1 Introduction

From a genetic standpoint, the British population is one of the most studied in the world, with many different studies sequencing or genotyping individuals from across the U.K. (e.g. [42, 52–54]). These studies have been primarily aimed at researching the genetic basis of disease, but have also been used to investigate population history, substructure and the relationship of different sub-populations in the U.K. to other European countries [42, 55, 56].

The U.K. is also an ethnically diverse country, with 13.8% of individuals belonging to ethnic minority groups (source: ONS survey). Groups of people from across the world have migrated to the U.K. at different periods in the previous three centuries, driven by the legacy of colonialism, the transatlantic Slave Trade and economic reasons. Despite this, the roughly 9 million ethnic

minorities within the U.K. remain relatively understudied in the context of genetics. For example, every one of the 27 papers in the GWAS catalog with “U.K. Biobank” in the title, and two others presently in the catalog curation queue, limited their analyses to subgroups described in various terms as “White British”, “British”, “European”, “White European”, “Caucasian” or “White” [57]. The primary reason for this is reasonable concerns over the confounding effect of population substructure within a cohort [58]; retaining a more genetically homogeneous cohort is one strategy to mitigate this.

Removing ethnic minorities from GWAS analyses is problematic, as evidence is mounting that the results from GWAS, including Polygenic Risk Scores (PRS), may not be transferrable to other populations if they have been conducted in cohorts of exclusively European individuals [59–61]. The reasons for this is currently unclear, but it has been suggested that differences in LD structure may be the cause [62]. Ethnic minorities may therefore miss out on the advances in healthcare driven by large-scale genomic projects.

Understanding the population structure of ethnic minorities within the U.K. Biobank is an important step towards including a diversity of ancestries in GWAS. Zaidi and Mathieson (2020) [63] showed that whilst it is not possible to correct for recent population stratification using principal components of common variants, correcting using a matrix of pairwise IBD sharing can. Similarly, it has been shown (S.Hu, personal communication of unpublished data) that principle components did not correct for GWAS hits on birth location, whereas using a ChromoPainter coancestry matrix could. This study painted the target samples using a reference and use the resulting painting profile as a reference.

Aside from the medical benefits, there is intrinsic value in studying the ancestry and population history of understudied minorities.

Whilst previous studies have explored the use of haplotype-based methods

to account for population structure, they have all focused on individuals of European descent. Large reference panels are not available for African individuals and differences in LD structure and genetic variation mean the applicability may be different to Europeans. However, recent studies have outlined the power of haplotype-based approaches in inferring the population histories of different African ethnic groups [64, 65].

Recently, a large dataset, hereafter referred to as the ‘Human Origins’, has become available. At the time of writing, it is the most detailed dataset of genotype data from African individuals in terms of the number of ethnolinguistic groups represented. Whilst the dataset contains individuals from across Africa, it contains particularly large numbers of individuals from South Africa ($n=104$), Cameroon ($n=567$) and Ghana ($n=211$), which are countries known to have contributed immigrants to the U.K. Therefore, this dataset is ideal for use as a reference panel to investigate the ancestry of ethnic minorities within the U.K. Biobank. In particular, given our newly acquired data come from parts of west Africa that may well represent sources of African ancestry among UK minority groups, I chose to investigate individuals with recent African ancestry. However, these results should in theory be equally applicable to other non-European populations, such as those from east and south Asia.

One potential issue is that only 70,776 SNPs overlap between the U.K. Biobank and Human Origins genotyping arrays. This is much lower than the number used in a typical ChromoPainter analysis, which is usually between 500,000 and 700,000. Using a low number of SNPs in the analysis may reduce the power to infer accurate ancestry proportions, in particular for haplotype-based methods since haplotype information depends on SNP density. Therefore, one option is to impute the non-overlapping SNPs using a reference panel. However, the effect of imputation on ChromoPainter-style analyses has yet to be fully investigated. It is possible that imputing a large number of positions may introduce biases, particularly towards populations which are present in the

reference panel. Studies have shown repeatedly that genotypes in non-European individuals are imputed less accurately compared to European individuals when using a primarily European reference panel [31, 66]. Accordingly, we can ask whether it is preferable to retain a smaller number of non-imputed SNPs or a larger number SNPs, some of which have been imputed.

This chapter will focus on two questions. Firstly, I am interested in relating the genetic variation of individuals in the U.K. Biobank with recent African ancestry to the populations in the Human Origins dataset, to try and infer their ancestral origins. Secondly, I will evaluate the effect of imputation on ChromoPainter analysis.

3.2 Results

3.2.1 4% of U.K. Biobank individuals have at least 50% non-European ancestry

Performing ChromoPainter analysis on the approximately 500,000 individuals in the U.K. Biobank would be computationally unfeasible; therefore I performed supervised ADMIXTURE on all 488,378 U.K. Biobank individuals. In order to identify individuals with at least 50% African ancestry, I set $K = 4$ supervision clusters that were defined using European (CEU), Gujarati, Han Chinese and Yoruban reference individuals. These individuals with more than 50% ancestry from Yoruba would then be carried forward to later ChromoPainter analyses.

In total, there were 8476, 2653, 9171 individuals with at least 50% inferred ancestry related to Yoruba, Han Chinese and Gujarati reference populations respectively, corresponding to 4.16% of the total U.K. Biobank individuals. Although I use these population labels for convenience, I note that an individual with e.g. 50% ‘Han Chinese’ ancestry does not necessarily derive 50% of their ancestry from the Han Chinese population, but that 50% of their ancestry

most closely matches Han China relative to the other reference populations. Thus, a Japanese individual may be modeled as 100% Han Chinese whilst not being Han Chinese in an ethnic sense. Similarly, for brevity, I will refer to individuals who have more than 50% of their ancestry from Yoruba as being ‘African’ Biobank individuals, whilst acknowledging ‘African’ as a broad label which encompasses a large diversity of ancestries and ethnicities.

I chose to validate the ADMIXTURE results to ensure that there has not been any mixing of individual labels and that enough iterations had been performed. To do this, I selected all individuals who self-identified as being either “Caribbean”, “African” or “Black or Black British” ($n=7527$) and assessed the distribution of ADMIXTURE ancestry proportions, under the assumption that these individuals should contain more African than other kinds of ancestry. This was the case, with the mean proportion of African ancestry among these individuals being 0.88 (Fig. ??), compared to 11 % British, 0.22% Han Chinese and 0.19% Gujarati.

However, there was substantial variation in the ancestry proportions for those who self-identified as being either “Caribbean”, “African” or “Black or Black British”. Proportions of Yoruban and British ancestry ranged from 0.00001 to 1, Han Chinese from 0.00001 to 0.53 and Gujarati from 0.759 to 0.00001, reflecting the diverse array of genetic ancestries that can fall under a given ethnic label. This suggests that relying on self-reported ethnicity may yield variable results when e.g. used as a covariate in a GWAS. For example, there were 48 people who self identified as being either “Caribbean”, “African” or “Black or Black British”, but had less than 1% African ancestry.

3.2.2 To impute or not?

In order to use ChromoPainter and the Human Origins dataset as a reference to infer fine-scale ancestry in U.K. Biobank individuals, the datasets must be merged. The overlap of SNPs genotyped in each dataset is only 70,776

SNPs, or ≈ 1 SNP per 40kb. Given linkage disequilibrium (e.g. as measured by Pearson's correlation) between pairs of SNPs decays to background levels by 100kb within most populations [67], analysing 70K SNPs may substantially decrease any potential power gains from modelling haplotypes to detect fine-scale differences between populations. In contrast, the imputed U.K. Biobank dataset has 535,544 SNPs in total, all of which are genotyped in the Human Origins reference dataset and 87.7% of which are imputed in UK Biobank individuals. While this may boost power over using only 70K SNPs, including a high percentage of imputed SNPs may bias ancestry inference. Therefore, I needed to determine a) whether there is a loss of power in the 70K SNP dataset and b) whether there is bias in the imputed dataset.

To answer these questions, and therefore determine whether using the imputed or the 70K SNP dataset is better in this scenario, I performed a painting using (i) the full 560,442 genotyped SNPs, (ii) 64,762 genotyped SNPs overlapping UK Biobank, and (iii) 500,000 SNPs that include the 70,000 genotyped SNPs and 430,000 SNPs imputed using the HRC reference. I performed painting (ii) in both linked and unlinked mode to determine whether there is haplotype information using 70,000 SNPs.

For each of the 3 datasets described above, I selected all ethnic groups from Nigerian, Cameroon and Ghana which had 5 or more individuals ($n=51$ pops, $n=1203$ individuals) and split each population randomly in half, into 'donors' and 'recipients'. I painted all recipient populations ($n=51$) using all donor populations ($n=51$) using a leave-one-out approach. I tested the information content of each painting by counting how often individuals copy more from individuals in their own populations than individuals from other populations. I also counted the number of times a population had the lowest TVD with its own population (Table 3.1).

Populations in the 70K painting matched more to and had a lower TVD to their own populations than the 500K painting. Whilst it seems counter-

painting	TVD	copying
70K (linked)	44%	24%
70K (unlinked)	20%	17%
imputed (linked)	14%	14%
full (linked)	38%	23%

Table 3.1: Percentage of populations which had lowest TVD (TVD) or copied the most (copying) from their own population under different paintings. 70K linked used 70,000 SNPs in linked mode, 70K used 70,000 SNPs in unlinked mode, imputed used 430,000 imputed and 70,000 non-imputed SNPs in linked mode and full used 500,000 non-imputed SNPs in linked mode.

intuitive that there is more power using a smaller number of SNPs, this is broadly consistent with my previous findings, which showed that metrics of painting information plateau after approximately 50,000 SNPs (i.e. there is no obvious benefit to using more than 50,000 SNPs). This is reassuring and suggests there is no loss of power when using the 70K SNP set. These data also shows that there is a loss of power when using imputed data relative to non-impute data.

Given the above results suggested that imputing data results in a loss of information, I was interested in whether this constituted a ‘bias’ towards certain populations. Imputation methods rely on identifying reference haplotypes which are closest to the target haplotypes. However, if the ethnic groups that the target individuals derive ancestry from are not present in the imputation reference panel, missing variants are imputed from populations in the reference panel which are most closely related to the target samples. In this case, two target populations would be imputed to appear more genetically similar to that reference population, reducing the differentiation between them. In theory, this artificial similarity would be propagated through to the ChromoPainter analysis. In particular, we would expect populations present in the reference panel to donate more to all other individuals than they would if no imputation had taken place.

For example, in the case of the Haplotype Reference Consortium, the closest

reference population to two African target samples from e.g. Cameroon may be the Yoruba from Nigeria, which is the only west African group in the reference. These samples would appear more similar to the Yoruba ethnic group than if they had not been imputed. In a ChromoPainter analysis, the Yoruba donor population would donate more than when using non-imputed SNPs.

Comparing the imputed and non-imputed coancestry matrices revealed biases consistent with the above expectation. If the coancestry matrix columns are combined into populations, then the sum of each column gives the total length of genome that population contributes to all recipient individuals in the dataset. Therefore, comparing the column sums between the imputed and non-imputed matrices informs us about which populations contribute more when using imputed compared to non-imputed SNPs. Fig 3.1 shows the amount of differential haplotype donation on a per-population basis, with populations highlighted based on their presence or absence in the 1000 genomes dataset. It is clear that populations present in the 1000 genomes are primarily clustered towards the right hand side, rather than randomly distributed across figure. This strongly suggests that imputation causes a bias towards those populations present in a reference panel.

Put together, these results suggest that using imputed data would introduce a level of bias and loss of information. Therefore, in all later analysis, I chose to use the approximately 70,000 non-imputed SNPs which overlap between the Human Origins and U.K. Biobank datasets.

3.2.3 African ancestry in the U.K. Biobank samples is concentrated in Ghana and Nigeria

Following from the previous section, I decided to use the dataset using approximately 70,000 unimputed SNPs. I performed a painting using all Human Origins individuals as donors and all U.K. Biobank individuals with at least

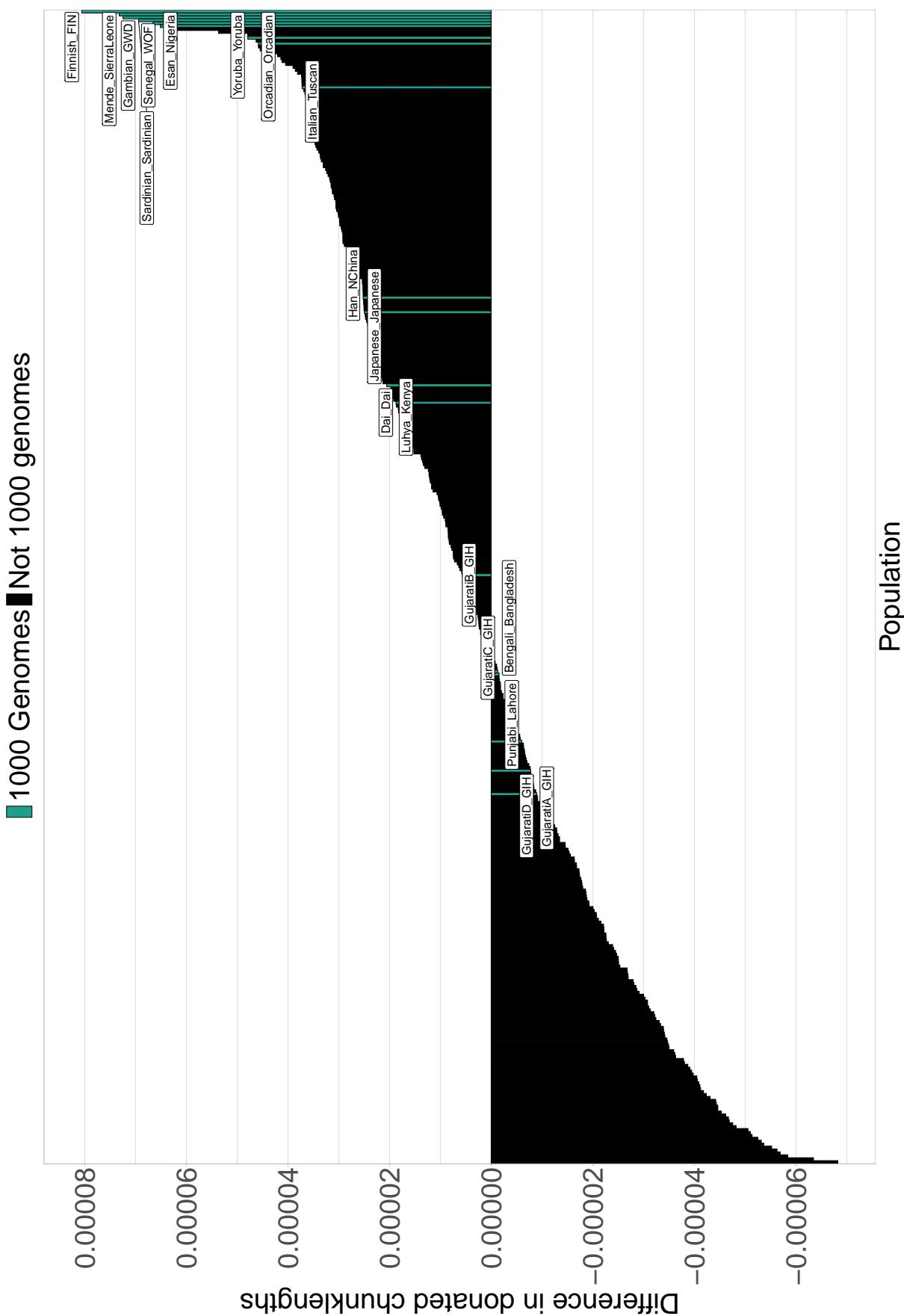


Figure 3.1: Differences in the amount donated by populations when using imputed and non-imputed data. Each vertical bar corresponds to a single population ($N=395$), with the height of the bar corresponding to the difference in haplotype donation, with positive values indicating increased donation and negative values indicating reduced donation. Bars coloured in green are also present in the 1000 genomes imputation panel and black bars are not present in the 1000 genomes.

50% African ancestry as recipients.

Principal component analysis on resulting chunkcounts coancestry matrix reveals the general structure of the selected individuals, alongside the reference populations (Fig. 3.2). Three clines can be observed; one of similarity to Southern African populations typified by the Zulu ethnic group from South Africa, one of similarity to West African populations such as Yoruba and Cameroon_Dii, and the last to East African populations such as those from Ethiopia, such as Ethiopia_Ari-Potter. The majority of U.K. Biobank individuals are positioned near West African populations; in particular between Yoruba and Cameroon_Arabe. The presence of a broad cluster of West African individuals is consistent with prior expectations that West African ancestry should be prevalent in a sample of British individuals. A second cluster of UK Biobank individuals is located along the Southern African cline, close to the Bantu_SA label.

Aggregating the columns of the co-ancestry matrix by reference population and taking the sum gives the total length of genome that donor population has contributed to the selected U.K. Biobank individuals. This can be visualised on a map, where each point represents a reference population and the colour corresponds to the total amount that reference population contributes towards the ancestry of all retained U.K. Biobank individuals (Fig. 3.3). Higher values correspond to more ancestry from that population in the U.K. Biobank sample. I will show in section 3.3.4 that this pattern is not a result of unequal donor population sizes.

The map supports the findings from the PCA in Fig. 3.2; the populations with the largest contribution are those from West Africa (Fig. 3.3). In particular, populations from Ghana and Nigeria contribute the most to the ancestry of Biobank individuals. On the other hand, populations in East and North Africa contribute relatively little, with Southern / South-East Africa being approximately intermediate. This is consistent with two different historical

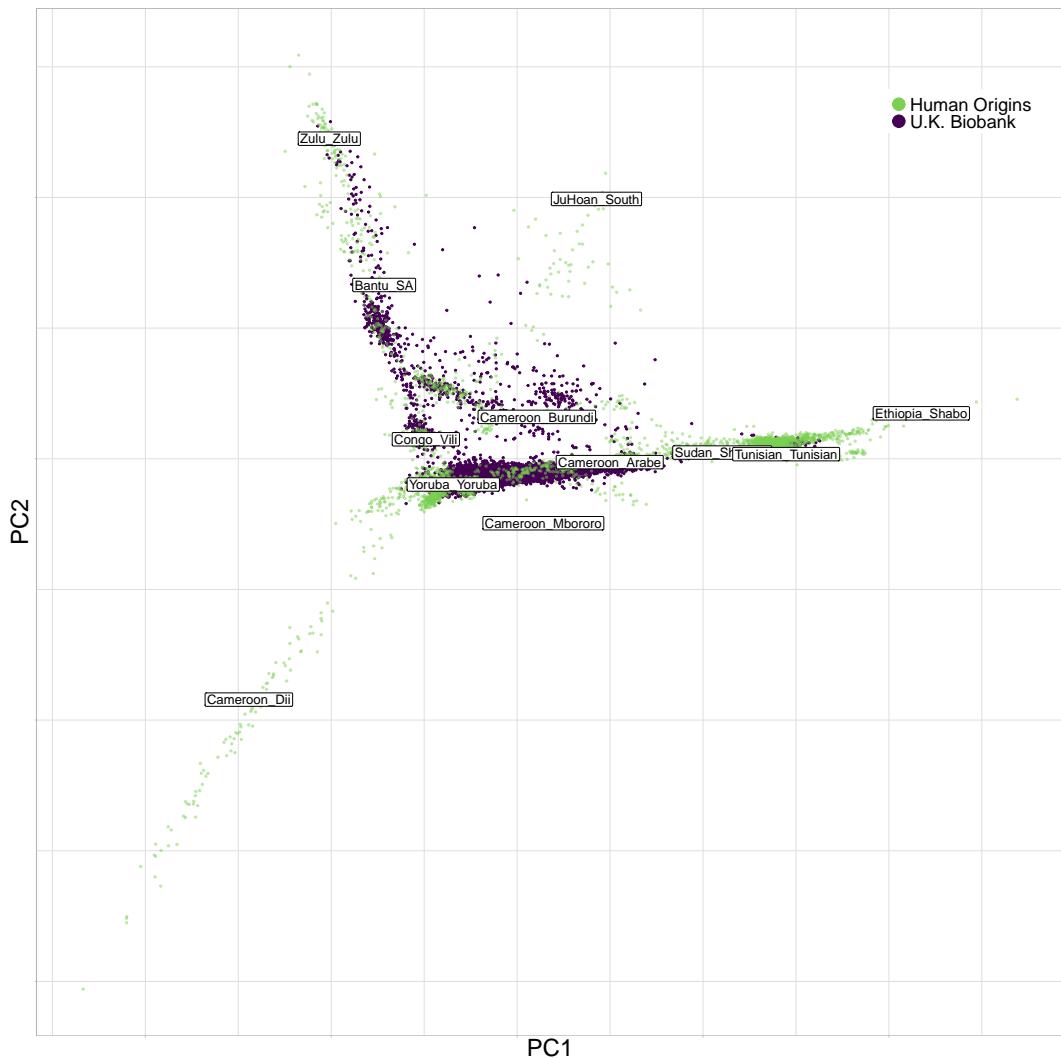


Figure 3.2: Principle component analysis of chunklengths matrix for all African U.K. Biobank individuals and human origins array. Individuals are coloured dependent on whether they are U.K. Biobank (green) or Human Origins (purple) samples. Labels indicate mean principle component coordinates for individuals in that population. A random sample of populations were chosen to have labels to prevent the figure from being too cluttered.

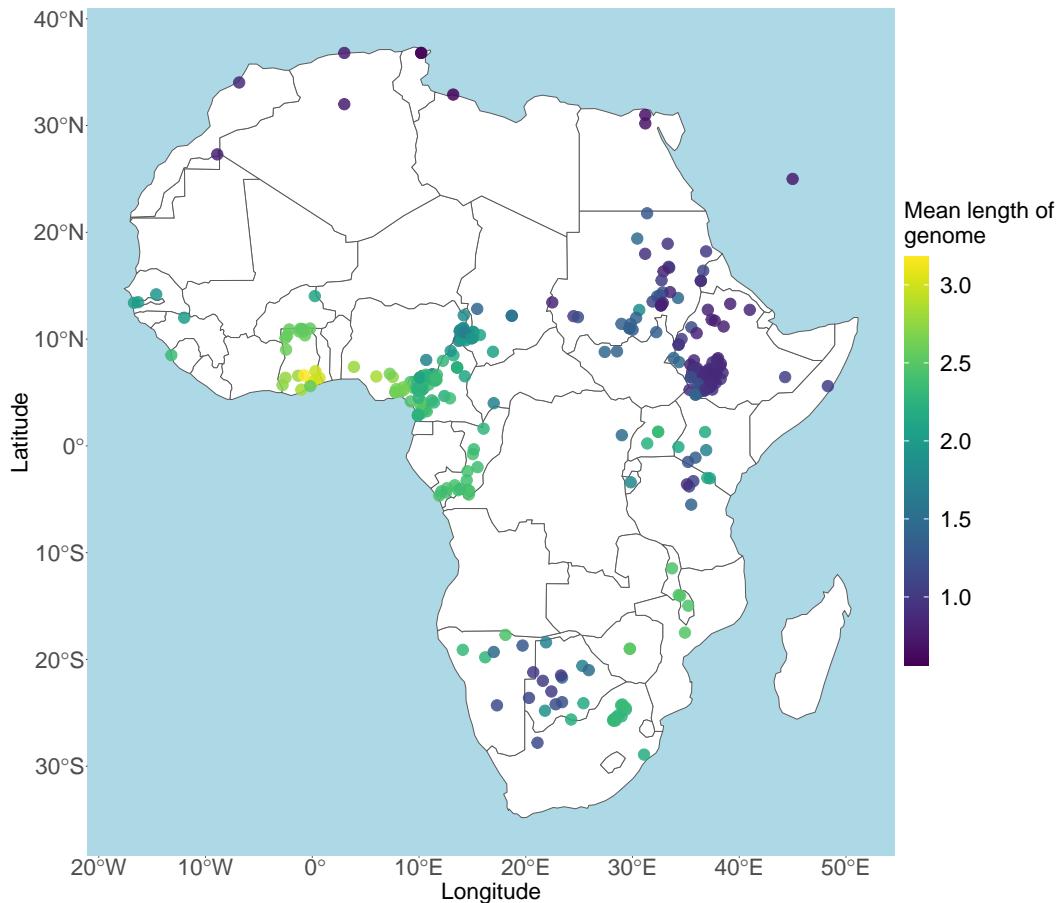


Figure 3.3: Map of haplotype donation to U.K. Biobank individuals. Each point represents a different African population. Colour corresponds to the mean length (cM) that population donated to all African U.K. Biobank individuals.

events.

Firstly, it is known from historical and genetic studies that a majority of the individuals who were forcibly transported from Africa to the Americas during the transatlantic slave trade were from the west coast of Africa [68]. Given the U.K. Biobank sample contains many individuals who were either born in, or trace their ancestry from the Caribbean, a region that had a large influx of slaves [69], we would expect there to be a large contribution of ancestry from West Africa. Secondly and more recently, there has been a relatively large amount of historical immigration from countries in West Africa, such as Ghana and Nigeria, to the U.K. Although there are a number of immigrants from other parts of Africa, reflected in the nonzero contributions from other ethnic groups, these contributions are small compared to those from West Africa [reference needed].

I performed the visualisation using the painting using imputed SNPs and the ancestry distribution was qualitatively the same.

I used SOURCEFIND to infer the proportion of ancestry that each UK Biobank individual shares most recently with each of the 535 surrogate groups, as this accounts for uneven donor population sizes. A map of proportions is given in Fig. 3.4.

Fig. 3.5 displays the 30 ethnic groups with the highest mean proportions of ancestry within the U.K. Biobank individuals. Yoruba was a clear standout for the most represented population; the mean proportion of Yoruba ancestry per individual was 40.0% and 3604/8309 individuals had at least 50% Yoruba ancestry. This is compared to the next most common ancestry, Ghana_Fante, which had an average of 7.3% per person and 373/8309 individuals with at least 50% ancestry. It is not clear what the reason for the large amount of Yoruban ancestry is. Interestingly, of all the individuals for which we have country of birth data for ($n=6190$), more of them were born in the Caribbean ($n=2263$)

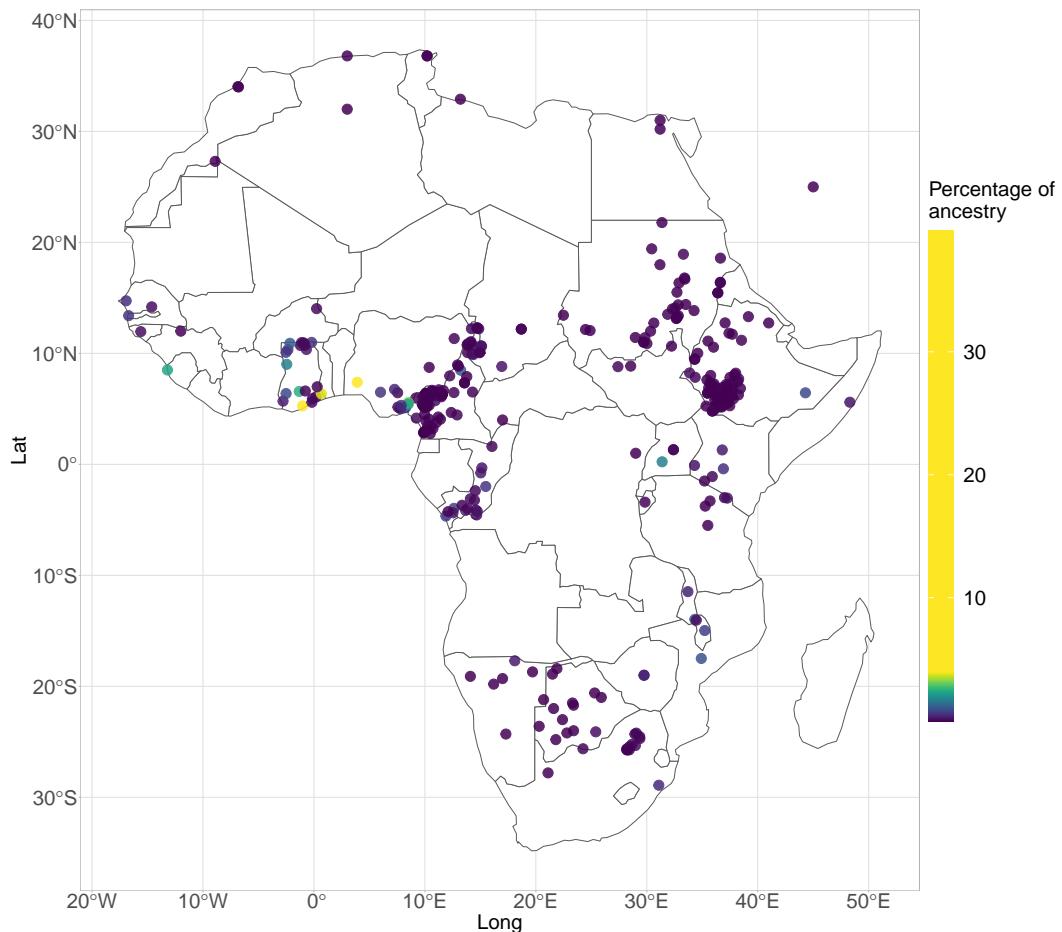


Figure 3.4: Map displaying the mean proportion of SOURCEFIND estimated ancestry of each African reference population within U.K. Biobank individuals. Each point is an African reference population with the colour corresponding to the mean ancestry proportion for that population across selected U.K. Biobank individuals. The colour-bar has been rescaled as two populations, Yoruba and Ghana_Fante have substantially higher proportions than all other populations.

relative to any other country; and of the individuals born in the Caribbean, over half were assigned to the Yoruban ethnicity. Therefore, one could tentatively explain the abundance of Yoruba ancestry as resulting from the transatlantic Slave Trade, where individuals from the Yoruba ethnic group were taken to the Caribbean at a higher frequency than other nearby ethnic groups in the Human Origins reference. The relatively large number of individuals from the Caribbean in the U.K. would thus have brought Yoruban ancestry to the U.K.

There are other instances of an over and under-representation of one ethnic group from a particular country (Fig. 3.6). For example, Uganda is dominated by a single ethnic group because we only have reference data from a single group in Uganda. On the other hand, the individuals from Sudan are more evenly distributed across ethnicities; however, this may be caused by an inability to distinguish individuals in closely related Sudanese populations.

Some other patterns can be noted. Whilst many individuals have intermediate levels of ancestry from West African populations (e.g. Ghana_Fante or Yoruba_Yoruba), much fewer individuals have intermediate levels of Ethiopia_Somali ancestry (Fig. 3.5). This may be because Somali individuals tend to be relatively unadmixed relative to West African populations and hence can be modeled as a mixture of almost entirely Ethiopia_Somali ancestry. Another option is that we have relatively fewer surrogate populations from East Africa and so any individual with East African ancestry is likely to copy a lot from Ethiopia_Somali, whereas West African individuals may have their ancestry spread across the many surrogate groups from West Africa [YOU CAN TEST THIS – DO PEOPLE WITH WEST AFRICAN ANCESTRY TEND TO HAVE LESS OR MORE TOTAL AFRICAN ANCESTRY RELATIVE TO THOSE WITH SOMALI ANCESTRY? IT SEEMS ANOTHER EXPLANATION IS THAT PEOPLE WITH W.AFRICAN ANCESTRY ARE ADMIXED (E.G. WITH EUROPE, DUE TO SLAVE TRADE) WHILE SOMALIS ARE RECENT MIGRANTS AND HENCE UNADMIXED.].

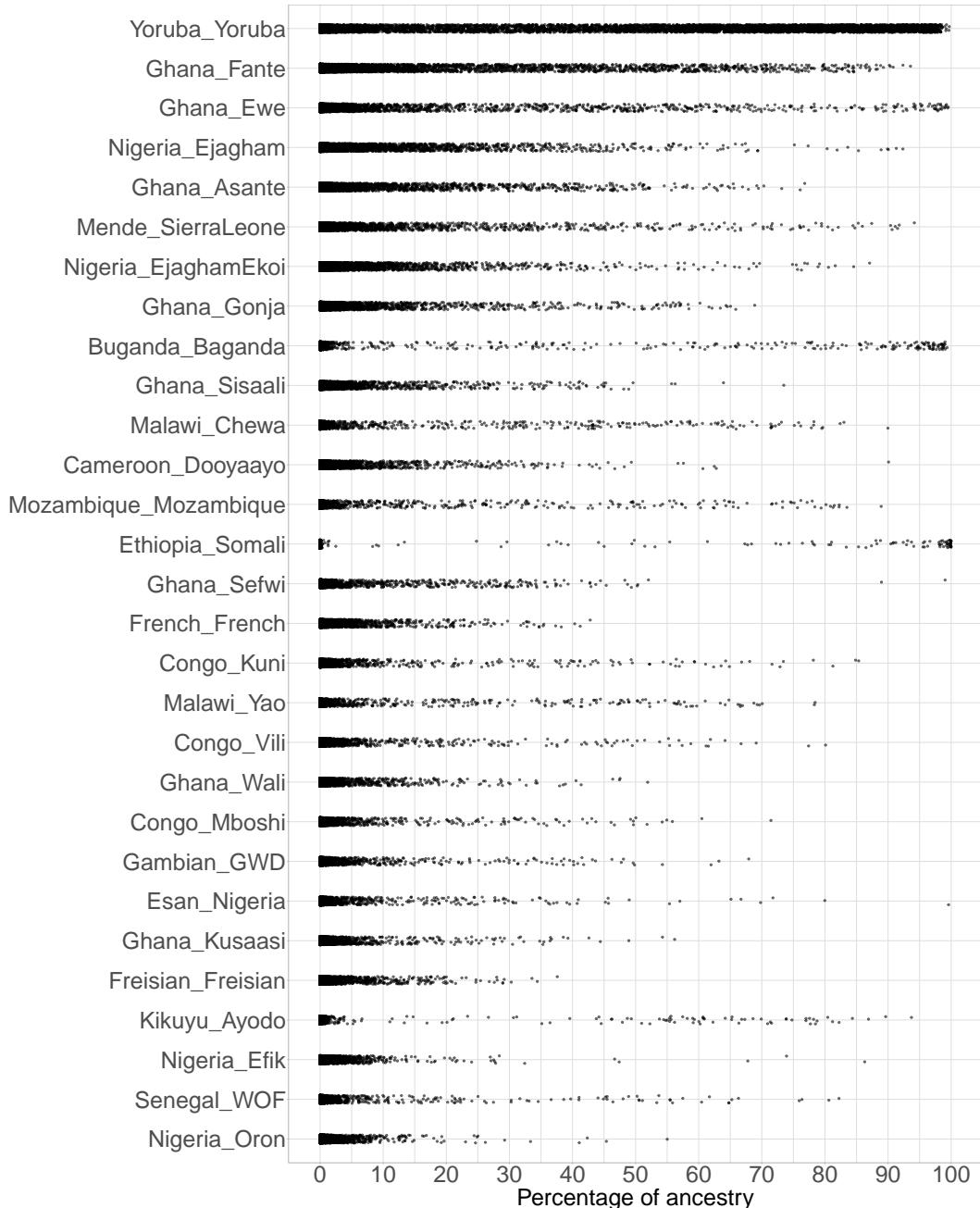


Figure 3.5: The 30 Human Origins populations which have the highest contribution to all U.K. Biobank individuals with at least 50% African Ancestry, based on SOURCEFIND analysis. Each row of points contains 8476 individuals and their position corresponds to the percentage of ancestry from that population.

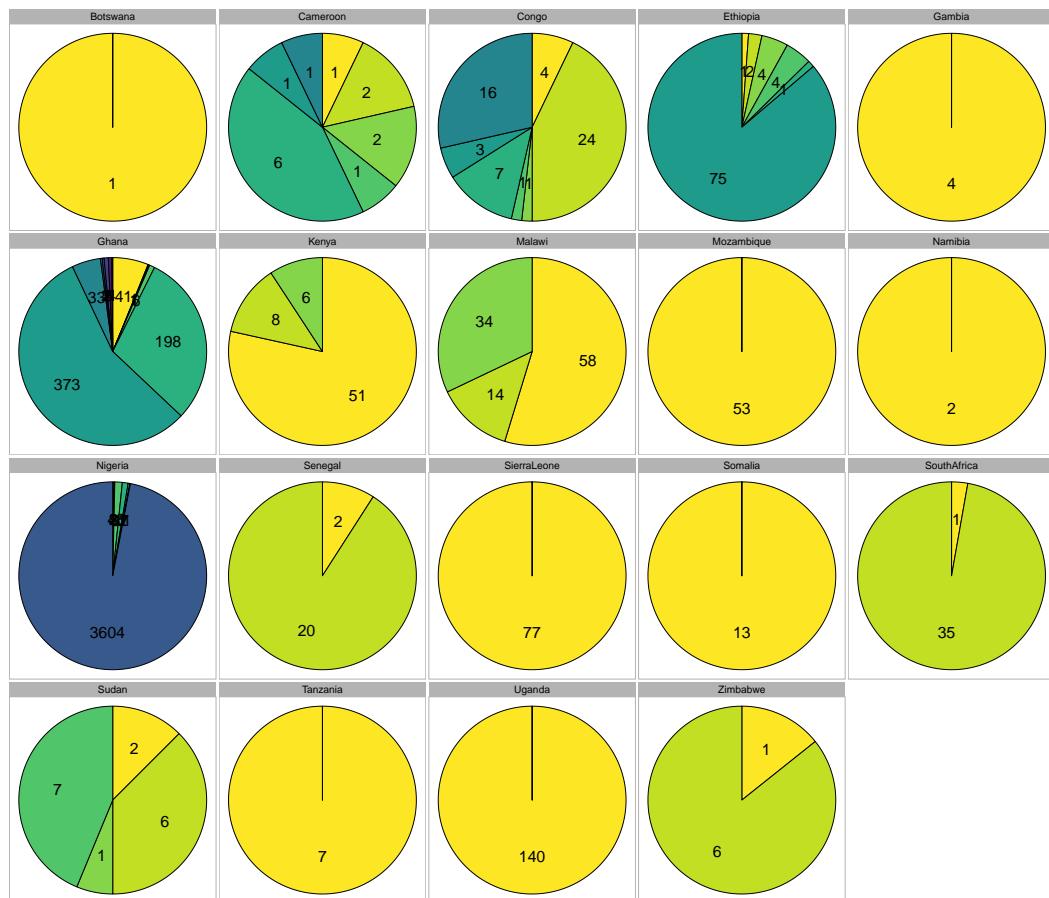


Figure 3.6: Variation of individuals assigned to different ethnic groups by country of assigned group. Each panel represents all individuals assigned to an ethnic group from that country, with proportions corresponding to different ethnic groups.

3.2.4 Verifying painting accuracy

Not all individuals within the U.K. Biobank were born in the U.K.; visualising the ancestry distribution of these individuals allows us to ensure that the painting is accurate and may reveal insights into population history. For instance, the ancestry distribution of individuals born in the Caribbean may provide evidence for the origin of slaves forcibly transported from the West Coast of Africa to the Caribbean during the transatlantic slave trade.

I subsetted the coancestry matrix to contain only U.K. Biobank individuals who provided data on birth location (6153/8472). We would expect that individuals who were born in a particular country would copy the most from reference populations from that country. For example, we would expect individuals who were born in South Africa to copy the most from sampled Bantu and Zulu ethnic groups from South Africa. This may not always be the case, as some ethnic groups have crossed borders in their history, but it should broadly be true. We also have birth place data for individuals who were not born in Africa (e.g. the Caribbean and Brazil).

Fig. 3.7 shows the map of haplotype donation from reference groups to U.K. Biobank individuals born in South Africa. It is clear that reference populations from South Africa, in particular the Zulu ethnic group, contribute the most to these individuals. The pattern is qualitatively the same for all countries which had a reasonable number of donor populations, suggesting that the painting had good resolution down to at least the level of individual countries.

There are several interesting results. For example, there are 2,263 individuals who were born in the Caribbean. Visualising the haplotype donation map for these individuals shows that they are primarily of West African ancestry (supplementary figure x) , consistent with historical evidence [68]. Individuals born in Brazil have ancestry from further South, again consistent with historical evidence (supplementary figure x). Of the 9 individuals born in Brazil, 6 of

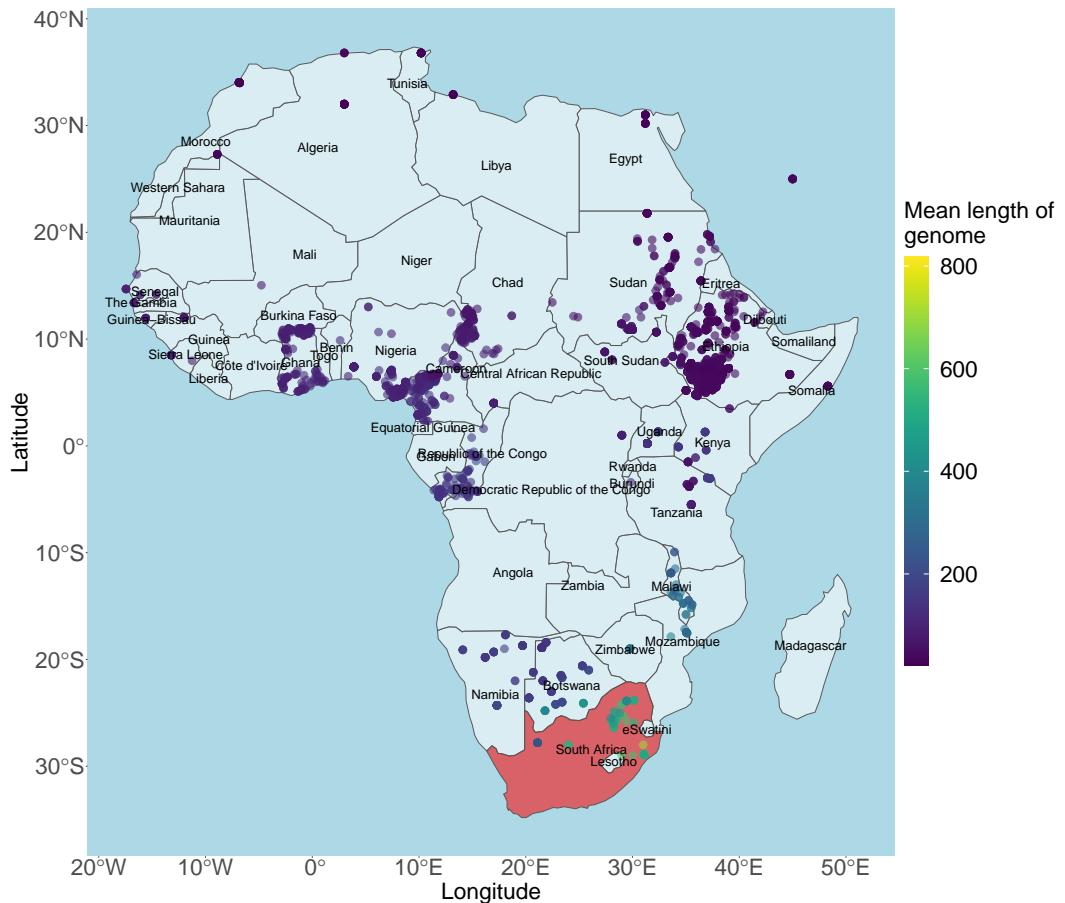


Figure 3.7: Map of haplotype donation to U.K. Biobank individuals born in South Africa. Each point represents one Human Origins population, coloured according to the summed amount of chunklengths that population donates to all U.K. Biobank individuals born in South Africa.

them had a majority SOURCEFIND component from an ethnic group in The Congo. However, it should be noted that there is a relatively small sample size from individuals born in Brazil ($n=9$), and that these individuals may not be representative of the Brazilian population.

As a formal test of the painting accuracy, I estimated SOURCEFIND ancestry proportions in each retained U.K. Biobank individual. An individual was ‘assigned’ to a particular ethnic group if they had 75% or more ancestry from that group. If the country the assigned reference population is from matches the birth location of the individual, then I considered that a ‘success’ and a ‘fail’ otherwise. Individuals who were born in the U.K. or who had no birth country were excluded from this analysis.

The overall accuracy at predicting birth location was 81.63%, suggesting there was substantial information within the coancestry matrix. For some countries where there was large number of surrogate populations, such as Ghana and Nigeria, the prediction accuracy was high. For certain countries, the prediction accuracy was much lower. For example, Tanzania, which is only represented by a single reference population, had a prediction match of XX%. Zimbabwe had by far the lowest prediction accuracy (14%) out of countries with more than 100 U.K. Biobank individuals. Of the 266 individuals born in Zimbabwe, 194 were assigned to an ethnic group from outside Zimbabwe; 74 to Malawi_Chewa, 71 to Mozambique_Mozambique and 49 to Malawi_Yao. Individuals from the ethnic groups from Malawi are found across Malawi, Zimbabwe and other countries, showing the possible weakness of this approach which aims to categorise individuals into a single country, as ethnic groups often transcend countries.

I also performed the same analysis but using the data which had been imputed. This stands as a practical test of whether it is preferable to impute or retain a smaller number of non-imputed SNPs. This yielded an accuracy of 81.89%, a value almost identical to that obtained with the dataset containing

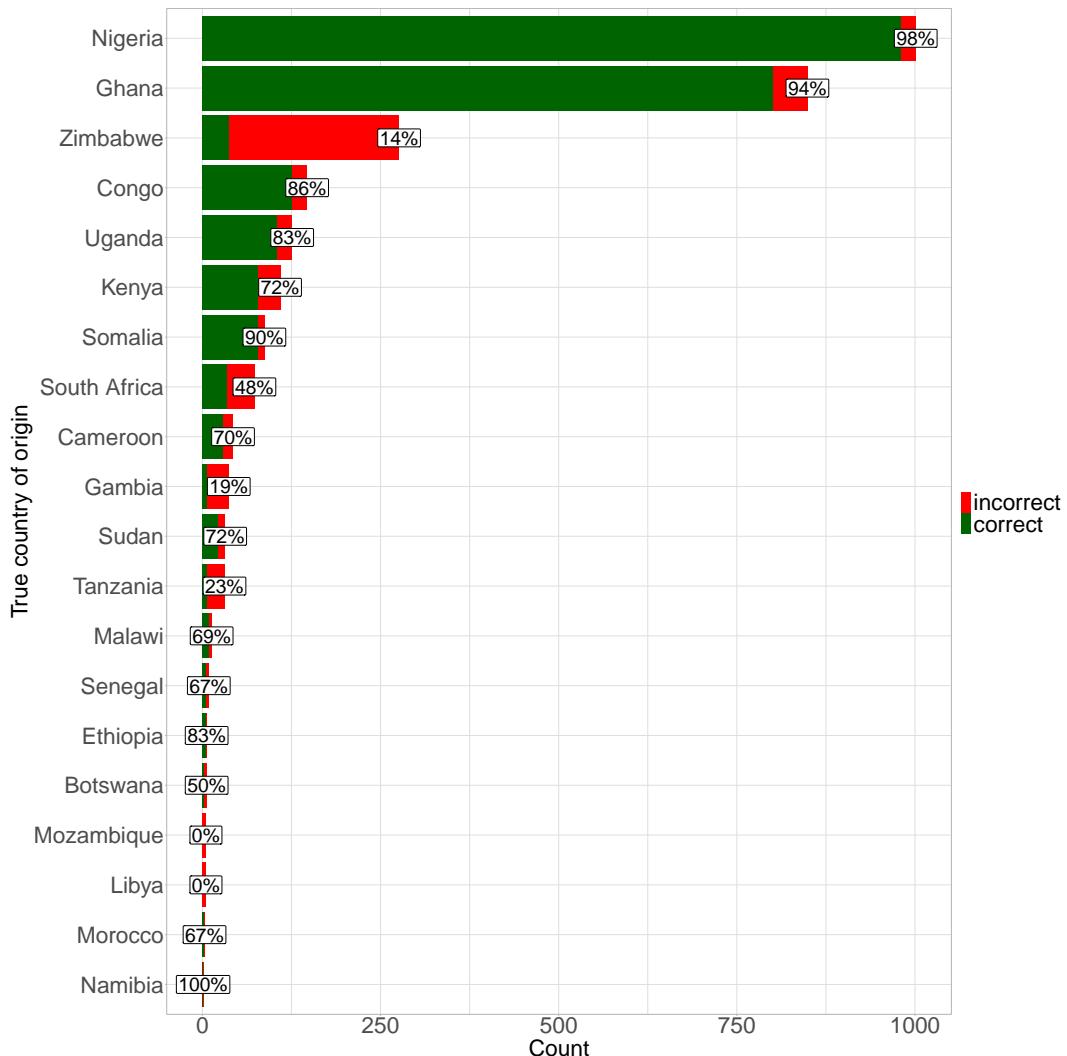


Figure 3.8: Correspondence of true birth country with estimated birth country. Each bar corresponds to a true birth country, with the length of the bar corresponding to the total number of people in our dataset born in that country. The green section corresponds to the total number of individuals where the birth country was correctly guessed and the red section to those who were incorrectly guessed. Percentage labels give percentage correct for that country.

approximately 70,000 non-imputed SNPs, despite my earlier results indicating that sub-country SOURCEFIND results are less accurate if using imputed data due to reference bias. This suggests that broad-scale ancestry assignment (i.e. assigning individuals to countries) is not affected by imputation, whereas sub-country estimates are.

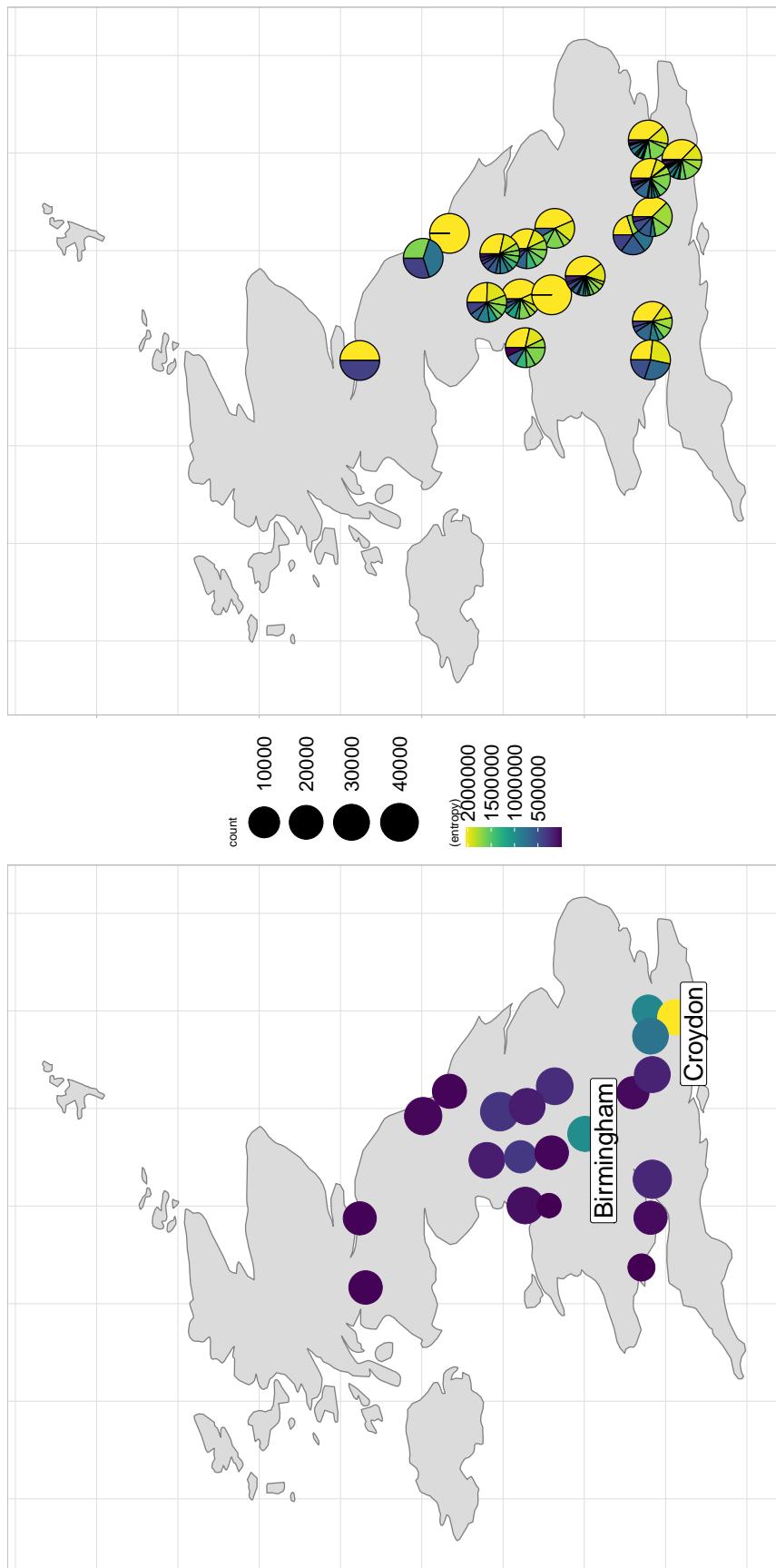
[WOULD ALSO BE GOOD TO KNOW WHERE THEY DIFFER; FOR WHICH POPS IF AT ALL (E.G. MAYBE A SCATTERPLOT OF ACCURACIES UNDER THE TWO CASES)]

3.2.5 Patterns of African ancestry across the U.K.

The U.K. Biobank dataset also contains data on the testing centre that each individual registered at. I used this information to see whether there was structure in how different ethnicities are distributed across the U.K. There was no apparent outliers in terms of centers and the proportion of individuals who had at least 50% African ancestry (Fig. 3.8). No clear pattern was apparent, other than Yoruba ancestry dominating most centres.

I also estimated the information entropy, E , of each centre based on the SOURCEFIND proportions, analogous to previous work done on European ancestry in UK Biobank individuals (S.Hu, personal communication). To calculate information entropy for each centre, I summed ancestry proportions for all 535 surrogate groups across all individuals from a centre, resulting in a vector, V of length 535. Then,

In this context, information entropy can be seen as analogous to the amount of diversity in different ancestries present in a particular centre. The two centres in the two largest metropolitan areas, London and Birmingham, have the largest amount of entropy [HOW MUCH SO? SHOULD HIGHLIGHT LONDON ON THE FIG – IS CROYDON A MISTAKE?], suggesting that ethnic diversity is greater in larger cities.



3.2.6 Patterns of African ancestry across the U.K.

Finally, I also had access to the birth-date of each U.K. Biobank participant. Therefore, it is possible to calculate the increase of the ancestry of a particular ethnic group over time 3.10. I took all U.K. Biobank individuals with more than 50% African ancestry and split them into 50 bins according to their birth date. Using a rolling window implemented using the `rollapply` function from the `zoo` R library, I calculated the mean age and mean proportion of Buganda_Baganda ancestry for each bin.

We can observe roughly a doubling of the mean proportion of Buganda_Baganda ancestry between 1950 and 1950.

3.3 Discussion

3.4 Methods

3.4.1 U.K. Biobank data access and initial processing

The U.K. Biobank dataset I had access to contains extensive phenotype data for 488,378 individuals and X phenotype measurements at the time of writing (<https://www.U.K.biobank.ac.U.K./>). Access was obtained to study the U.K. Biobank dataset via UCL Genetics Institute (ref number 51119, principal investigator = D.Curtis).

I obtained the U.K. Biobank genotype data, consisting of 488,377 individuals genotyped at 784,256 genome-wide SNPs on the U.K. Biobank Axiom Array. I will hereafter refer to these data as the ‘non-imputed’ data. I used plink1.9 [70] to convert the binary plink files to .bcf format.

I also obtained U.K. Biobank data which had already been imputed to approximately 96m SNPs, using the combined references of the Haplotype

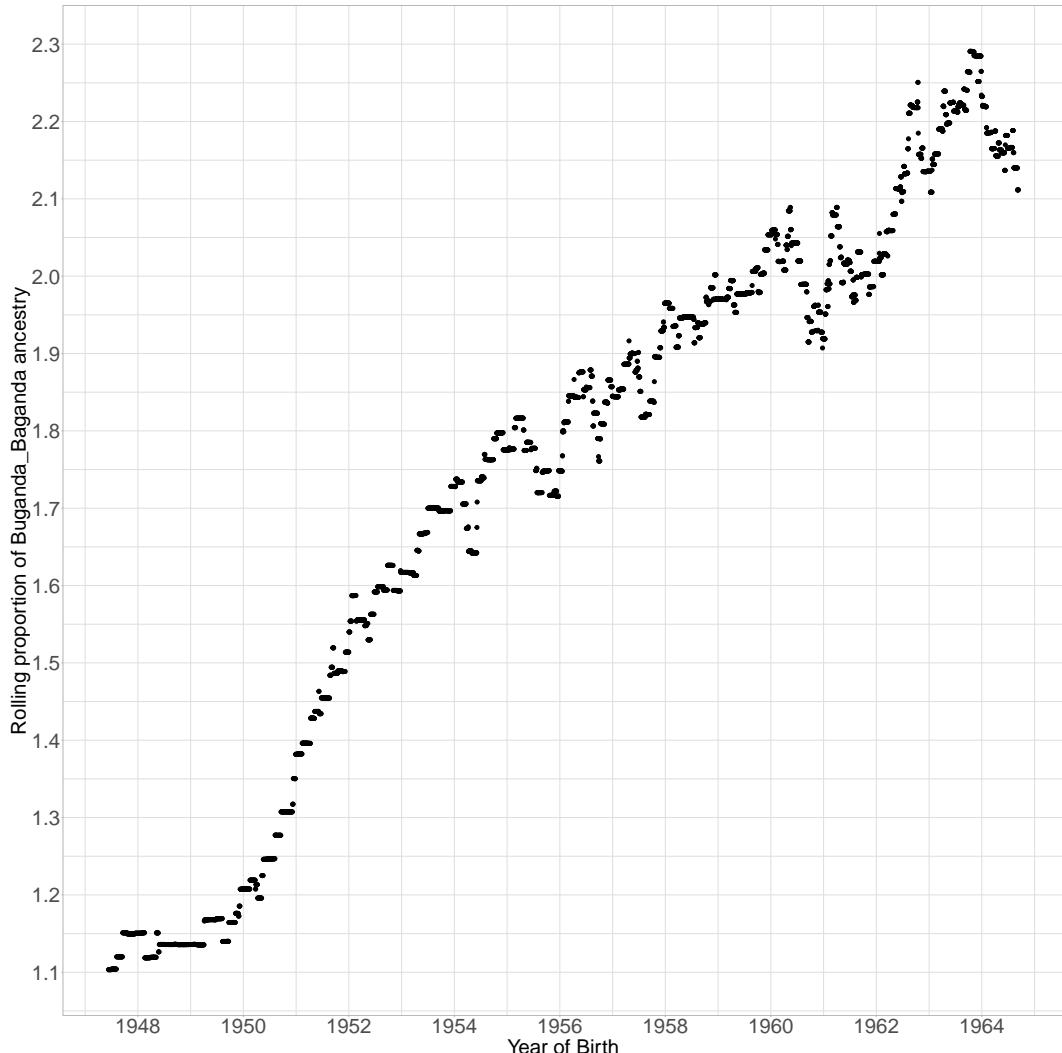


Figure 3.10: Correspondence of true birth country with estimated birth country. Each bar corresponds to a true birth country, with the length of the bar corresponding to the total number of people in our dataset born in that country. The green section corresponds to the total number of individuals where the birth country was correctly guessed and the red section to those who were incorrectly guessed. Percentage labels give percentage correct for that country.

Reference Consortium (HRC) and UK10K haplotype resource. Full details of imputation can be found in the paper of McCarthy et al (2016) [33]. The imputed data was downloaded and converted from `.bgen` to `.bcf` format using `qctool2` (https://www.well.ox.ac.U.K./~gav/qctool_v2/).

I therefore had two separate datasets; ‘imputed’ and ‘non-imputed’, containing the same individuals and differing only in whether or not imputation had been used to increase the total number of SNPs.

3.4.2 ADMIXTURE analysis

I am primarily interested in using ChromoPainter [9] to explore the ancestry of ethnic minorities in the U.K. Biobank[SHOULD JUSTIFY WHY? I.E. REALLY YOU’RE LOOKING AT WHETHER WE GAIN ANYTHING FROM USING HAPLOTYPE INFORMATION]. However performing ChromoPainter analysis on the entire U.K. Biobank dataset (n=488,377 individuals) is computationally infeasible. Thus, I chose to analyse only those individuals with more than 50% non-European ancestry. ADMIXTURE is a fast and accurate way to estimate continental-scale ancestry proportions [41] and is therefore ideal for this task.

An alternative option to using ADMIXTURE would be to use PBWT (positional Burrows-Wheeler transform) paint <https://github.com/richarddurbin/pbwt/blob/master/pbwtPaint.c>), a fast approximation to ChromoPainter which provides approximately the same output and is scalable to large sample sizes [71]. However, it is not possible to provide a reference panel and each haplotype must be compared to all others in turn. This would be much less efficient and would not allow me to take full advantage of the Human Origins dataset.

I LD-pruned the non-imputed U.K. Biobank dataset using using `plink -indep-pairwise 50 10 0.02` [70]. This left a total of 70,776 bi-allelic SNPs. I then subsetted the 1000 Genomes dataset down to the 70,776 SNPs retained

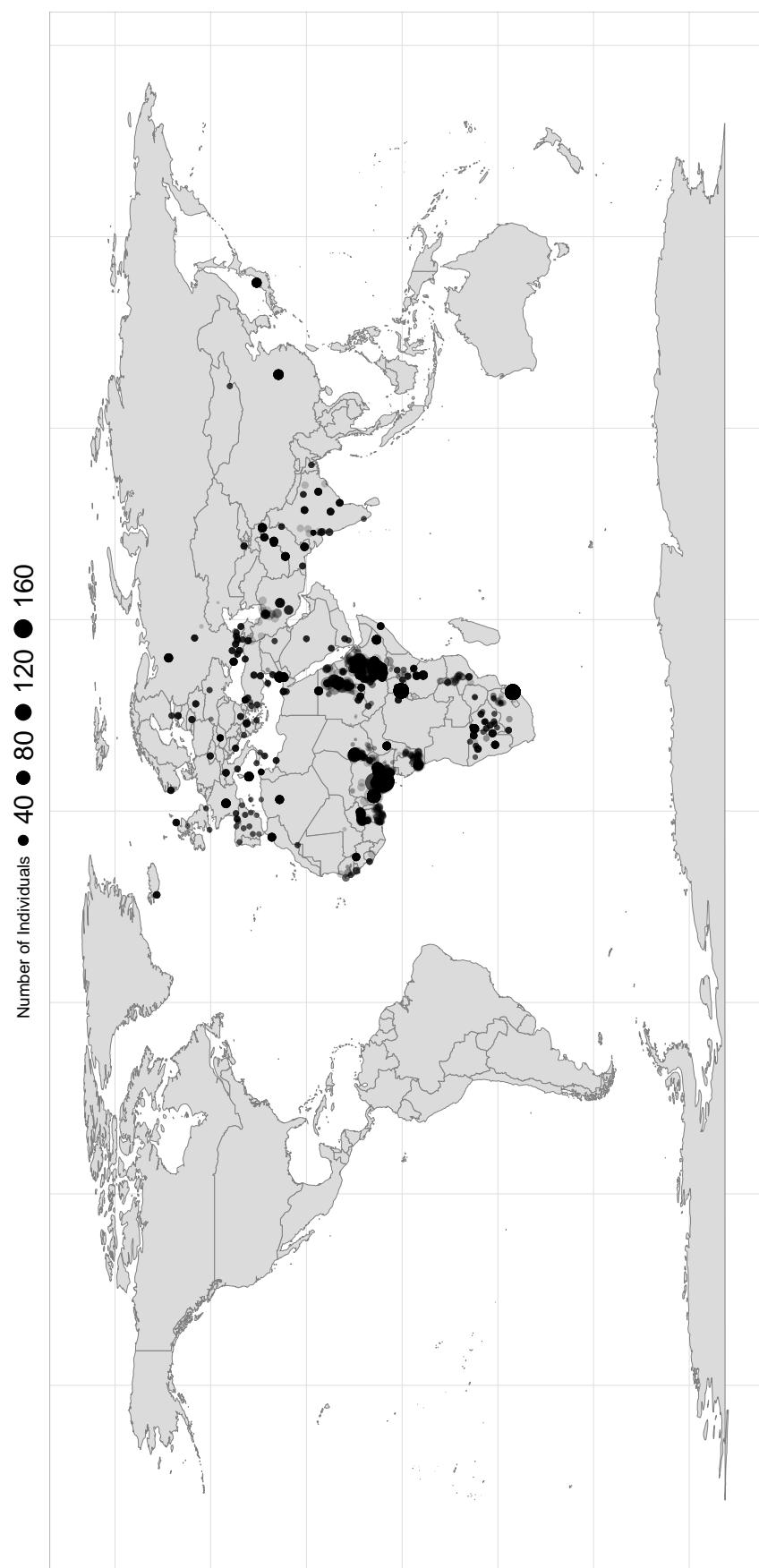
in the U.K. Biobank dataset and merged the two datasets using `bcftools -merge`. Thus, I had a dataset containing all U.K. Biobank and 1000 Genomes individuals, genotyped at 70,776 SNPs.

I ran ADMIXTURE in supervised mode using the argument `-supervised` and fixed the 4 reference populations as GBR British, Nigeria Yoruba, Han Chinese and Gujarati Indian from the 1000 Genomes dataset. These populations were chosen as they represent a broad division of worldwide populations into African, European, East Asian and South Asian; for the purposes of this particular piece of analysis, it was not necessary to include finer-scale populations. The rest of the arguments were left to default. I used the resulting `.Q` files to determine the ancestry proportions of each reference population in each U.K. Biobank individual.

Individuals with at least 50% ancestry from Nigeria Yoruba were carried into later analysis; I refer to these as ‘selected’ Biobank individuals.

3.4.3 Data preparation - Human Origins

To determine the ancestry of U.K. Biobank individuals, I compared their SNP patterns to people from different parts of the world to infer which populations they share recent ancestry with. As I am particularly interested in studying individuals with recent African ancestry, I used the so-called “Human Origins” reference dataset (appendix A.20) for this purpose, as it contains individuals from 349 different ethnic groups from across Africa and 535 world-wide groups in total (Fig. 3.11. Full details of processing can be found in Appendix A.20 (Human Origins dataset).



3.4.4 Data merge - non-imputed data and Human Origins

I used `bcftools -merge` to merge 5,998 reference “Human Origins dataset” individuals with 8,476 UK Biobank participants that had $\geq 50\%$ African ancestry, using the gt-conform utility from Beagle (<https://faculty.washington.edu/browning/conform-gt.html>) to remove any inconsistent positions. This dataset contained 65,749 non-imputed SNPs that overlap between the Human Origins and UK Biobank arrays. I phased these data with shapeit4 [31] using `-pbwt-depth 8`, the b37 genetic map and otherwise default parameters.

3.4.5 Data preparation - imputed data

I similarly merged the imputed UK Biobank data with the Human Origins reference dataset at 525,566 SNPs that were typed in Human Origins, and phased these data with shapeit4 in the same manner.

3.4.6 Chromopainter

For each of the imputed and non-imputed datasets, I used CHROMOPAINTER to infer the proportion of genome-wide DNA that each UK Biobank and Human Origins reference individual matches to individuals from each Human Origins reference population. Using this CHROMOPAINTER output, I then used SOURCEFINDv2 [11] to infer the proportion of ancestry that each UK Biobank individual shares most recently with each of the XX Human Origin reference populations

3.4.7 SOURCEFIND

I estimated ancestry proportions for each of the selected U.K. Biobank individuals using SOURCEFINDv2 [11]. I used the combined painting from the section above. I analysed each U.K. Biobank individual with more than 50%

African ancestry separately, using all Human Origins populations as surrogates. I left all parameters as default.

3.4.8 Imputation bias test

The imputed U.K. Biobank dataset was imputed using a reference panel containing the Haplotype Reference Consortium. Whilst this contains many European populations, it contains relatively few from Africa. Imputing variants in non-European individuals using a reference panel that is primarily composed of European individuals may lead to biased or inaccurate imputation. Given I am particularly interested in analysing individuals with recent African ancestry in the U.K. Biobank, it is important to determine whether this is the case. A natural test case would be to compare a painting on the U.K. Biobank individuals using imputed and non-imputed SNPs. However, this would not be possible as the imputed dataset contains approximately 500,000 SNPs, whereas the non-imputed dataset only contains 70,000 SNPs. Having an order of magnitude difference in the number of SNPs means it would not be possible to disentangle the combined effects of SNP count and imputation on the estimates of bias.

Thus, I used the Human Origins dataset, where I could control a) the imputation and b) the total number of SNPs used in the analysis. I submitted the full Human Origins reference dataset (5998 individuals and 560,420 SNPs) to the Sanger Imputation Server (<https://imputation.sanger.ac.U.K./>), which uses the full Haplotype Reference Consortium (HRC) as a reference panel for imputation. This reference panel was chosen because it was the same one used for imputing the U.K. Biobank individuals.

I next subsetted the imputed Human Origins dataset down to SNPs present in the U.K. Biobank array, leaving 727,325 positions present in the imputed Human Origins dataset. Although the number of SNPs still differ, my previous research (cite figure) shows that increasing the number of SNPs beyond 400,000

does not affect the ability to correctly assign individuals to populations from a painting. I phased the imputed and unimputed datasets separately using shapeitv4. For each dataset, I then used CHROMOPAINTER to form each phased haploid of the 5998 Human Origins dataset individuals as a mosaic of all other phased Human Origins dataset haploids, using the ‘all-v-all’ approach.

Therefore, I had two co-ancestry matrices, that contain the inferred proportion of DNA that each HO dataset individual shares most recently with each other HO dataset individual of identical donors and recipients, but one was generated using imputed SNPs and the other was generated using no imputed SNPs.

Chapter 4

Bavaria ancient DNA

4.1 Introduction

Throughout the Pleistocene and Holocene, Germany has been the setting for many population movements and admixture events of modern humans. The Swabian Alps is home to one of the earliest symbolic art, dated to at least 32kya [72] and musical instruments dated to 40kya [73], both assigned to the Aurignacian tradition. Later, the region was also home to one of the first Neolithic traditions in the *Linearbandkeramik*, a key culture in the Neolithisation of Europe.

Cherry-Tree cave (Kirschbaumhöhle in German) represents an unique opportunity to study the transect of samples from the Neolithic to the present-day. The cave represents a relatively untouched layer of stratigraphy.

In the present-day, Germany represents a boundary point between East and West Europe; [???]current population structure [PRESUMABLY YOU'RE ADDING DETAILS, + REFS, HERE? THERE IS SOME INFO HERE IN LESLIE ET AL 2015 FIGURES]. Questions remain as to the origin of this East-West structure; is it recent structure, or does it persist to the Middle Ages or earlier?

Here, I present novel data from 11 medium-to-high coverage samples from two sites from Southern Germany and one site from one from Southern Austria. In particular, the samples from Kirschbaumhöhle span from the Late Neolithic to the Iron Age, providing an opportunity to study a time transect in a narrow geographic region.

Previous studies into the genomic history of Bavaria have focused, for example, on the mixed ancestry of migrant females during the Early Middle Ages.

A collaborator, Prof. Joachim Burger, Johannes Gutenberg University Mainz, posed the following 3 questions.

1. **Second Neolithic immigration wave.** One of the samples is thought to have belonged to the first wave of farmers carrying farming technology from the near-east to Europe, and another to the second. Do we observe genetic differences between the two waves of samples?
2. **Cherry Tree Cave.** How can we make sense of the genetic ancestry changes from the Late Neolithic through to the Iron Age in Cherry Tree Cave? Do we see evidence of genetic continuity between the ages and are they characterised by admixture from outside sources?
3. **Germanic / Slavic divide.** Is there a distinction between the Germanic and Slavic Middle Age samples? How do these populations compare to the preceding samples from the Bronze and Iron ages?

4.2 Methods

4.2.1 Data generation

Eleven whole-genomes of ancient individuals were generated by collaborators at the Johannes Gutenberg, University of Mainz, Germany. Their estimated

radiocarbon dates range from 1060AD to 5200BC (Fig. 4.2). Six of the samples were found in Cherry-Tree Cave in the Bavarian district of Forchheim (Fig. 4.1), four from futher South in the region of Dingolfing/Essenbach and one sample from southern Austria. The samples had a median coverage of 4.84x and ranged from 0.7x to 17.52x. Full details of coverage, location and dates are given in Table 4.1.

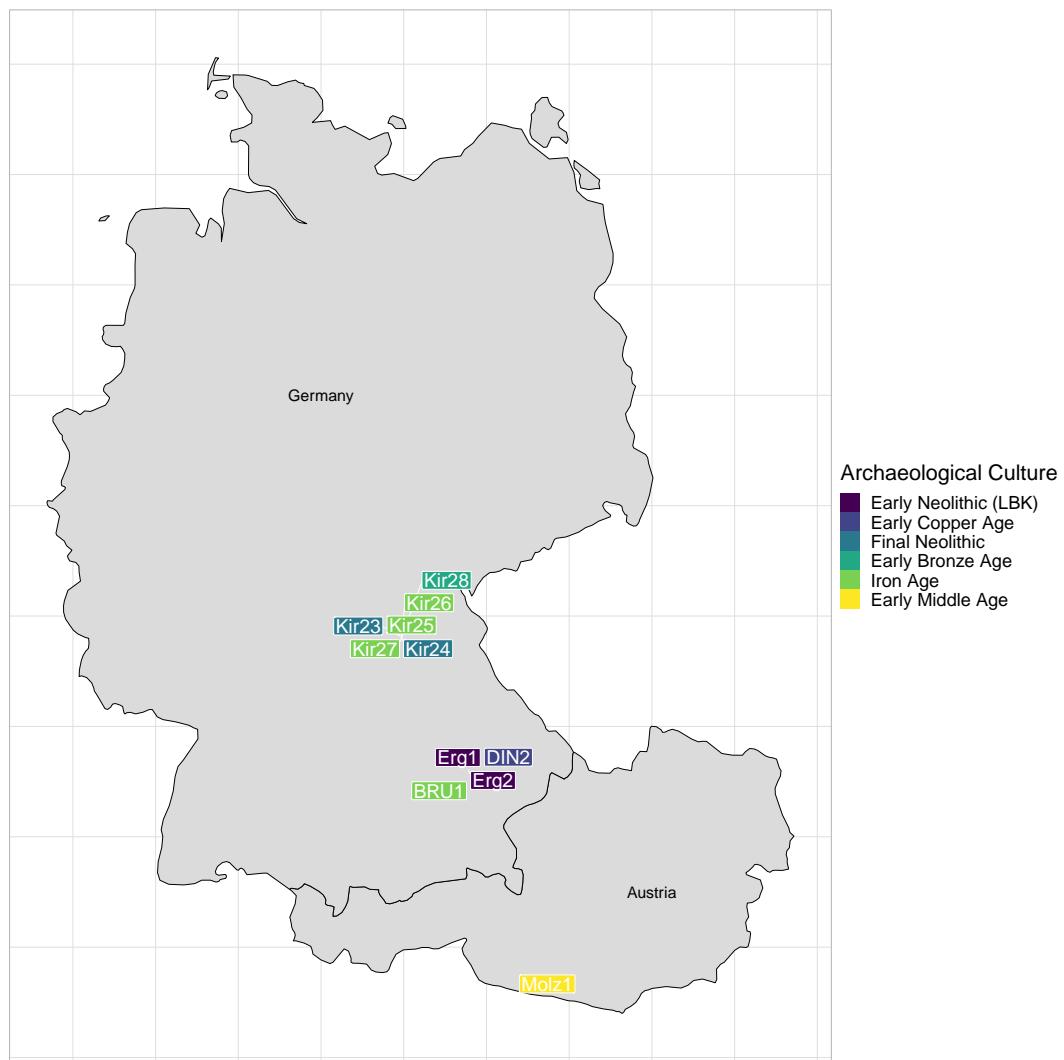


Figure 4.1: Map of newly sequenced ancient individuals, positioned according to where they were excavated. Colour on label corresponds to archaeological culture which they were found.

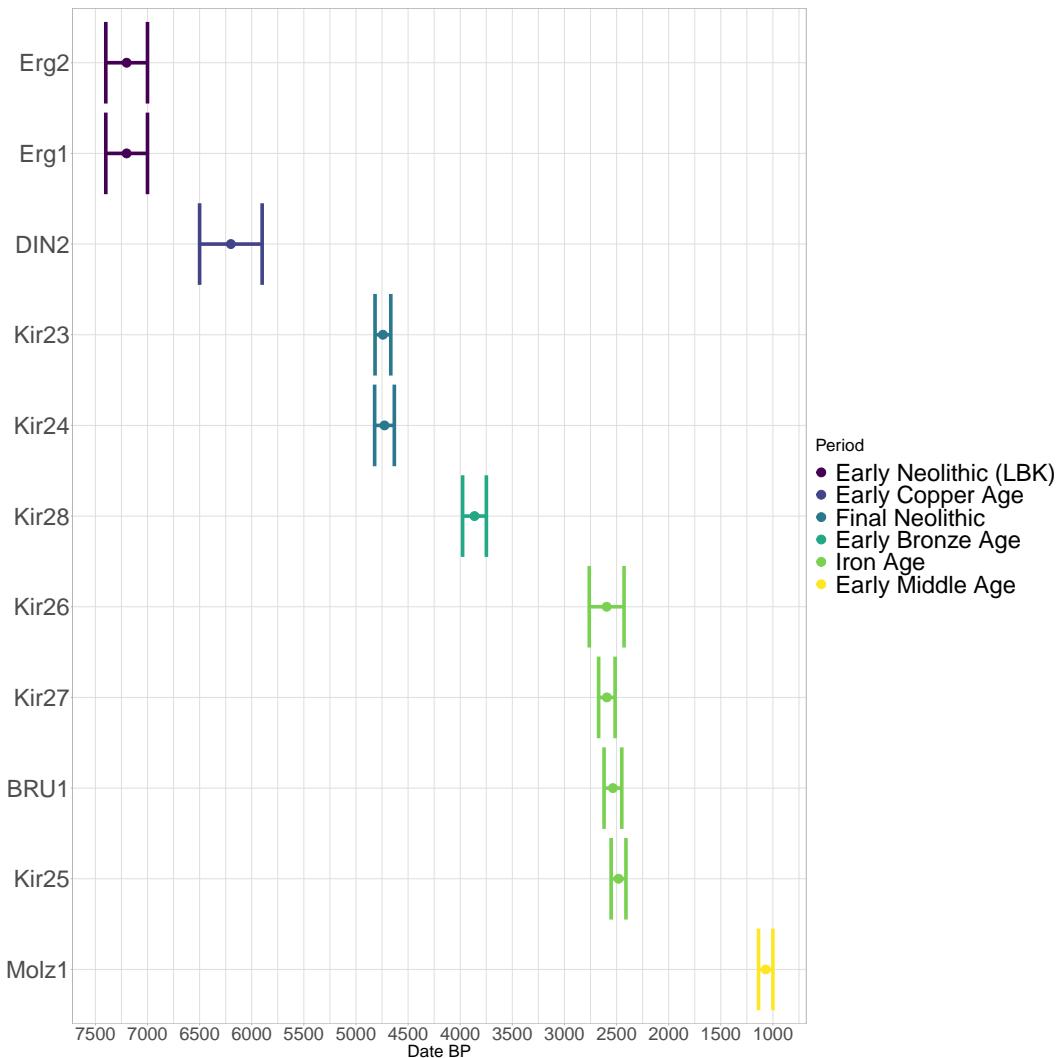


Figure 4.2: Estimated radiocarbon dates for each newly sequenced ancient individual, grouped by archaeological period.

Sample.ID	Location	Date	Period	Coverage
Erg1	Ergoldsbach-Essenbach	5200	Early Neolithic (LBK)	4.52
Erg2	Ergoldsbach-Essenbach	5200	Early Neolithic (LBK)	0.71
DIN2	Dingolfing	4200	Early Copper Age	1.71
Kir24	Cherry Tree Cave	2762	Final Neolithic	3.98
Kir23	Cherry Tree Cave	2741	Final Neolithic	17.52
Kir28	Cherry Tree Cave	1863	Early Bronze Age	17.30
Kir26	Cherry Tree Cave	595	Iron Age	4.84
Kir27	Cherry Tree Cave	593	Iron Age	16.60
BRU1	Bruckberg	535	Iron Age	11.54
Kir25	Cherry Tree Cave	481	Iron Age	4.55
Molz1	Molzbichl	1069	Early Middle Age	13.22

Table 4.1: Table providing details for the newly sequenced Bavarian samples.

4.2.2 Stuff that Jens did (e.g. read aligning)

Collaborators in University of Mainz performed DNA extraction, read alignment and variant calling. [OBVIOUSLY HAVE TO CHANGE TITLE, AND ADD MORE DETAILS.]

4.2.3 Genotype imputation and phasing using GLIMPSE

I merged the 11 newly sequenced individuals with the reference data-sets A.1 to A.17 resulting in a total of 942 individuals in .bcf format with genotype likelihood data at 77,213,942 genome-wide SNPs. Data was then split into separate .bcf files for each chromosome and indexed using bcftools [74].

As before, I followed the recommended GLIMPSE [24] imputation and phasing pipeline (https://odelaneau.github.io/GLIMPSE/tutorial_b38.html), here using the 30x-coverage 1000 genomes dataset [35] as a reference when phasing and imputing data in these 11 newly sequenced individuals and 942 individuals from published datasets A.1-A.17 in Table XX. I used default settings to split the genome into windows for efficient imputation/phasing, in the end generating phased samples with calls at 50,342,061 total bi-allelic autosomal SNPs.

4.2.4 Determination of uniparental haplogroups

I used Haplogrep (<https://haplogrep.i-med.ac.at/>) to identify the mtDNA and y-chromosome haplogroups for each newly sequenced ancient samples [75] from the raw .fastq files.

4.2.5 IBD sharing

I used hap-IBD [76] to estimate IBD segments between all pairs of ancient individuals, using the phased output from GLIMPSE as input haplotypes, the genetic maps from (<http://bochet.gcc.biostat.washington.edu/beagle/>

`genetic_maps/plink.GRCh37.map.zip`) and default parameters. I estimated IBD segments for each chromosome separately and summed their length segments between each pair of individuals across all chromosomes.

4.2.6 plink PCA

To obtain a broad overview of the ancestry of the newly sequenced individuals in the context of 915 other ancient samples, I performed PCA on the pre-imputation genotypes using plink2. Performing a PCA in plink2 allows for both an understanding of genome-wide variation patterns and the identification of any data quality issues that are independent of phasing or ChromoPainter analysis.

I retained the 500,000 markers with the lowest amount of missingness across all samples and LD-pruned the resulting SNPs using the settings `-maf 0.01` and `-indep-pairwise 50 5 0.2`. PCA was performed using default settings from plink2.

4.2.7 Chromopainter analysis

To characterise of the ancestry of the newly sequenced ancient samples in the context of other ancient individuals, I first selected all ancient samples above 1.5x coverage ($n=466$) and performed an ‘all-v-all’ painting where each phased haploid was matched to all other phased haplotypes. 1.5x was somewhat arbitrarily chosen as my previous work has shown this is a suitable threshold for the inclusion of samples for ChromoPainter analysis (section 2.5.4). I used this painting, hereafter referred to as ‘ancient’ painting, to perform fineSTRUCTURE clustering and tree building on ancient samples.

I performed Principle Component Analysis on the coancestry matrix of the ‘ancients’ painting using the `prcomp_irlba` function from the `irlba` R libary. To account for the fact that the diagonals of the coancestry matrix are always

Population	nsamples
HB:tsi	196
HB:spanish	68
HB:bulgarian	62
HB:german	60
HB:french	56
HB:russian	50
HB:greek	40
HB:ukrainian	40
HB:croatian	38
HB:hungarian	38
HB:norwegian	36
HB:southitalian	36
HB:polish	34
HB:romanian	32
HB:mordovian	30
HB:cypriot	24
HB:northitalian	24
HB:lithuanian	20
HB:siciliane	20
HB:westsicilian	20
HB:belorussian	18
HB:tuscan	16
HB:irish	14
HB:scottish	12
HB:germanyaustralia	8
HB:welsh	8

Table 4.2: Name of population and number of samples used in the present-day ChromoPainter analysis

zeros (as an individual cannot be painted by themselves), I set the diagonal of each row to be the mean of that row. Although there were 466 individuals in the ‘ancients’ painting, not all of these were included in the chunklengths PCA. This was because many individuals in that set were not relevant to exploring the ancestry of the Bavarian individuals. For instance, when plotted, samples such as those from the Xiong Nu, a 3rd century BC culture from inner Mongolia, dominate the variation in a PCA to the point where identifying structure between the samples of interest becomes challenging. Therefore I removed XX individuals based on visual inspection of the first two principal components

To determine the genetic similarity between the newly sequenced ancient samples and present-day populations, I performed an ‘all-v-all’ painting using a selected group of 26 present-day European populations (Table 4.2) from the HellBus dataset (described in appendix section XXX) plus the newly sequenced ancient individuals, hereafter referred to as ‘present-day painting’.

I applied fineSTRUCTURE (v0.0.5) [9] to cluster the chunkcounts ChromoPainter output for the ‘ancient’ painting. This algorithm assigns individuals to genetically homogeneous clusters, estimates the ‘true’ number of clusters and builds a dendrogram of genetic similarity[based on a tree-building algorithm. This is particularly useful when combining many samples from different studies, as is the case with the ‘ancients’ painting; the population label identifiers used by different studies may not be consistent with one another. Therefore, we can use fineSTRUCTURE groupings as population labels rather than group labels. fineSTRUCTURE was first run in MCMC mode using 1,000,000 burn-in MCMC iterations and 2,000,000 main MCMC iterations. It was then run in tree-building mode (`-m T`) using 100,000 burn-in and 100,000 main iterations.

Tree figures, co-ancestry matrix figures and principle component plots were generated using the fineSTRUCTURE R library (<https://people.maths.bris.ac.uk/~madjl/finestructure/FinestructureRcode.zip>).

4.2.8 SOURCEFIND

I used SOURCEFIND [11] to infer the proportions of ancestry by which each target (e.g. ancient) individual is most related to a set of surrogate populations. While this method does explicitly attempt to identify admixture, in contrast to (e.g.) ALDER [77] or GLOBETROTTER [10], it can reflect admixture proportions [11] but more generally reflects recent ancestry sharing patterns.

The first analysis used the ancients painting and only three surrogates: Western Hunter-Gatherers, Neolithic farmers and Yamnaya. This analyses

reflects previous research suggesting most ancient Europeans, with the exception of some paleolithic Hunter-Gather populations [49], descend from the mixture of three sources well-represented by these groups. The second analysis attempted to characterise more fine-scale ancestry patterns, by modeling each target ancient individual (using the same ancients painting) as a mixture of all sampled ancient populations that had an average sample age no more than 100 years younger than that of the target individual. The third analysis used the “modern” painting and formed each ancient individual as a mixture of all present-day populations shown in Table 4.2. For each of these analyses, I found the mean and 95% credible interval of ancestry estimates across 2,000,000 posterior samples combined from three independent SOURCEFIND runs that each sampled every 10,000 MCMC iterations after discarding the first 10,000 MCMC iterations as “burn-in”.

4.2.9 MOSAIC admixture analysis

I inferred admixture events, dates and proportions using MOSAIC, a haplotype-based method [78]. While MOSAIC cannot infer multiple pulses of admixture from the same admixing sources as GLOBETROTTER can in theory, it is unlikely we have power to identify such multiple pulses when analysing only a single ancient sample. Furthermore, the ‘painting’ step and admixture inference step in MOSAIC are combined, providing a simpler pipeline and more flexible assignment of different surrogates relative to GLOBETROTTER (i.e. the set of surrogates can be changed without repainting the samples).

I performed two different kinds of admixture analysis. First, I performed an ‘ancient surrogates’ analysis where the all ancient samples above 1.5x coverage were used as surrogates to admixing sources. I used the fineSTRUCTURE groupings to categorise ancient samples into surrogate populations.

I also performed a ‘present-day surrogates’ analysis where a selected set of present-day populations were used to analyse both present-day Slavic popula-

tions and ancient Slavic populations. While using present-day populations to reflect ancestry patterns in ancient individuals may be counter-intuitive, the larger sample sizes and larger variety of present-day populations can provide more refined results relative to using ancients

I ran MOSAIC using default settings, assuming two or three admixing sources per target individual/population. For populations with more than one sampled individual, MOSAIC provided bootstrap-based x% confidence intervals around date estimates. MOSAIC also estimates f_{st} between the set of surrogates and the estimated ‘true’ mixing source, which is useful when a close proxy for the ‘true’ mixing source is not available

4.2.10 F-statistics

Many of the relevant samples in the literature were of either very low coverage (< 0.1) or genotyped on a capture array. As my work in chapter 2 indicated that samples with less than 0.5x coverage cannot reliably be analysed using ChromoPainter, I also used F-statistics [13] that are mostly robust to coverage related effects [79]. In particular I used Admixtools (<https://uqrmaie1.github.io/admixtools>) to analyse 942 individuals from 143 populations (Appendices A1-A18), including many low-coverage samples from LBK cultures from Rivollat et al (2020) that would not have been suitable for use with ChromoPainter [80]. This analysis also incorporated 2280 present-day individuals from 144 populations from the HellBus dataset as putative ancestry surrogates for tested ancient individuals.

For the input to ADMIXTOOLS, I used the genotyped imputed from GLIMPSE, as it has been shown that using imputed markers reduced reference bias relative to using pseudo-haploid markers [17]. I then used the f_4 branch test to test whether two populations form a clade relative to two other populations. For example, the expected value of $f_4(french, german; yoruba, mbuti)$, which tests whether {french,german} form a clade relative to {yoruba,mbuti}, should

not give a score significantly different to zero. In contrast, exchanging *french* with *yoruba* would yield a significantly positive f_4 scores, with strength of evidence to reject the null ($f_4 = 0$) measured using standardised Z -statistics.

I also used the f_3 test, denoted $f_3(A, B; C)$, to (i) estimate the branch length between A and B after their divergence from C , or (ii) test whether C descends from an admixture event between sources represented by A and B . The latter can occur if C has a substantial number of SNPs with allele-frequencies which are intermediate between A and B .

Finally, I used qpAdm to infer ancestry proportions, following the protocol described in Olalde et al (2018) by choosing the following populations/samples as outgroups: *Mota*, *Kostenki14*, *papuan*, *han*, *hannchina*, *mbutipygmy*, *sannamibia*, *yakut*. These outgroups were suitable for use in investigating ancient Eurasians, since they are asymmetrically related to many ancient populations, but do not show evidence of recent gene flow with them.

4.3 Results

4.3.1 Broad-scale ancestry changes in Bavaria reflect those found elsewhere in Europe

As expected, on the first two principal components samples from the Early Neolithic (approx 5200BC) and Copper Age (approx 4200BC) cluster with other samples from the European Neolithic (Fig. 4.3). Previous studies have explained the pattern observed when Neolithic samples are plotted on a PCA [81]; the earliest Neolithic samples, from Anatolia and Greece, who are thought to be the source population from which all subsequent Neolithic farmers derive [16, 82–85], are usually positioned at the end of the cluster which is farthest away from the hunter-gatherer samples (for example, WHG on Fig. 4.3). This likely reflects the fact they are unadmixed with respect to the later

Neolithic samples. As the Neolithic progressed, farmers from the near-east mixed with local hunter-gatherer groups in central Europe [81] and acquired local hunter-gatherer ancestry. Accordingly, these samples are shifted away from the earlier Neolithic samples towards the hunter-gatherers. With this in mind, the position of Erg1, shifted north away from the contemporaneous sample Erg2, is suggestive of hunter-gatherer admixture.

There are four key observations from the ancient PCA regarding the new samples:

1. The two Late Neolithic individuals are genetically separate, with Kir24 positioned close to Yamnaya and Kir23 clustering with Neolithic Europeans.
2. The Bronze Age sample Kir28 clusters with other European Bronze Age samples
3. The four Iron Age samples (Kir25, Kir26, Kir27 and BRU1) cluster towards the Neolithic individuals and other European Iron Age samples
4. The three Medieval period samples (Alh1, Alh10, Molz1) cluster with the Bronze Age sample Kir28 instead of the Iron Age samples.

4.3.2 Early Neolithic

The 3 early/middle Neolithic samples all display a strong affinity to Anatolian farmers, consistent with the prevailing theory that near-eastern farmers were responsible for the spread of early agricultural technology across Europe, and that all Neolithic farmers share recent common ancestry [16,83–85]. fineSTRUCTURE analysis groups Erg1 with 2 samples from Upper Paleolithic/Neolithic Italy and DIN2 with Early/Middle Neolithic samples from Germany, Greece, Anatolia and Hungary. Despite their age, the genetic variation of the Early Neolithic samples falls well within the variation of present-day individuals; when

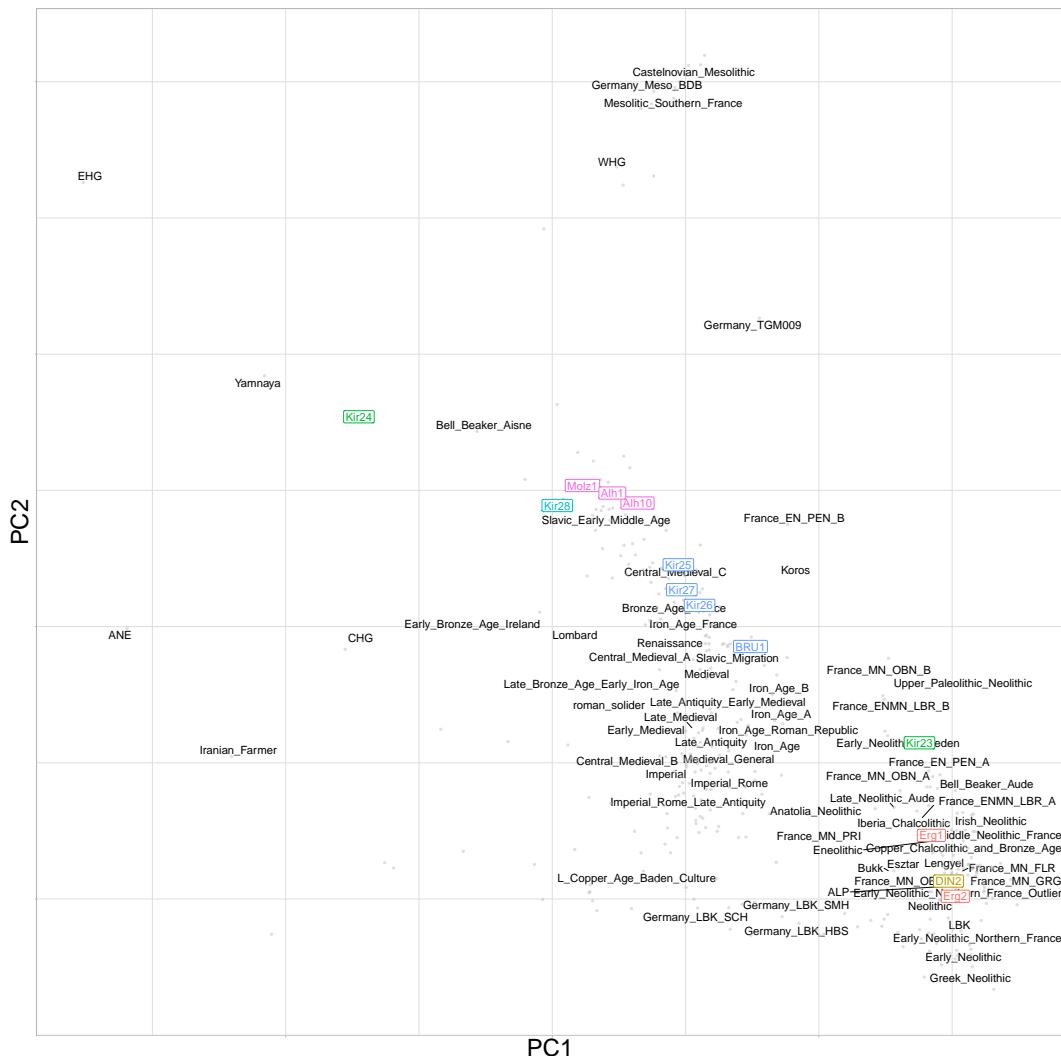


Figure 4.3: Principle component analysis of genotype matrix using plink2. Grey points indicate principle component coordinates for each sample. Black text indicated mean principle component coordinates for all individuals within that group. Coloured labels represent newly sequenced ancient samples.

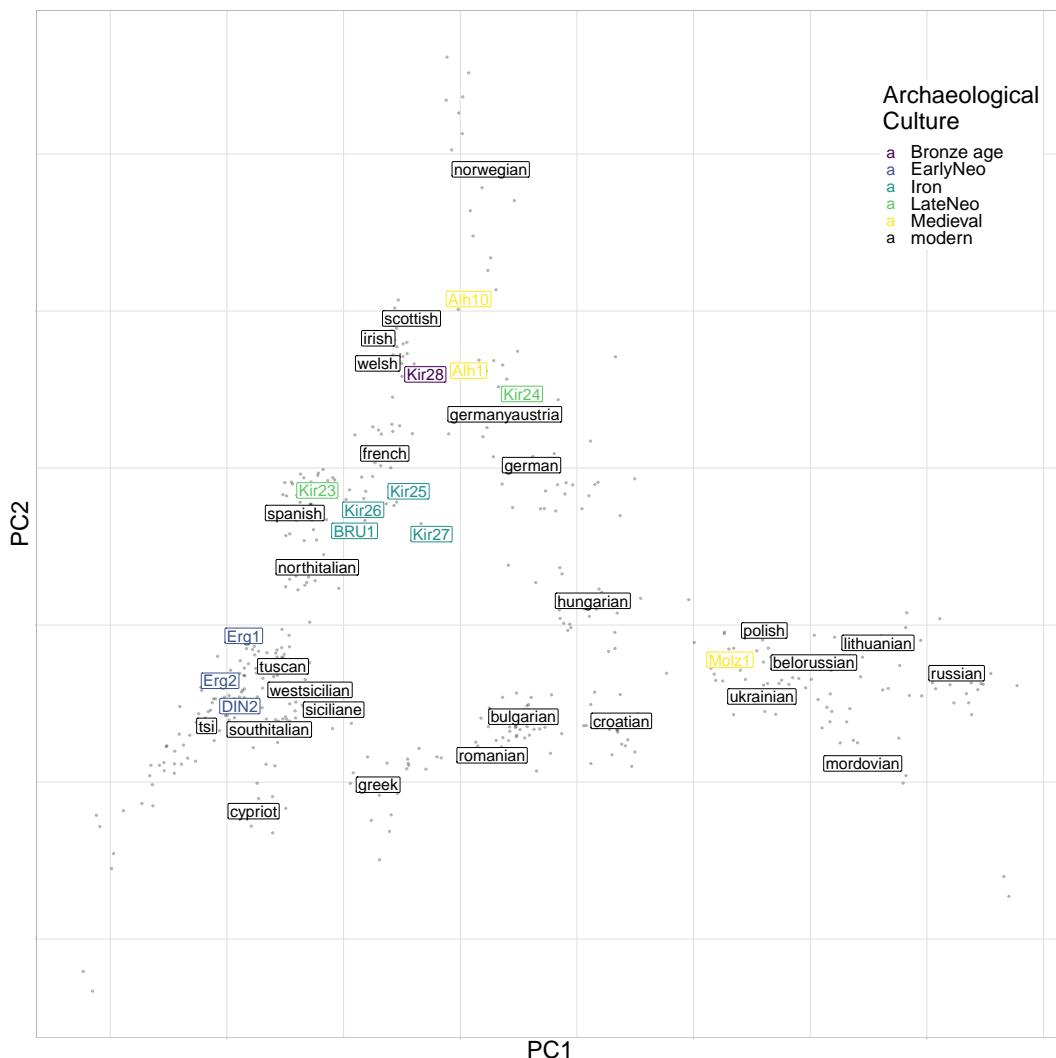


Figure 4.4: Principle component plot of newly sequenced ancient samples and reference modern individuals performed using the finestructure library. Green labels correspond to Migration Era samples, red labels correspond to Early Middle Age samples and white labels correspond to reference populations. The position of each reference label is the mean PC coordinates of all individuals within that population. Transparent coloured points correspond to present-day individuals.

painted using present-day samples, the 3 Early Neolithic individuals cluster with present-day Italians, consistent with findings from previous research [16,40] (Fig. 4.4. Erg1 was assigned to mtDNA haplogroup K which has been found in Neolithic and pre-pottery sites across Europe [82,86] and Western Asia [87,88].

Erg1 is from the *Linearbandkeramik* (LBK) culture and is speculated to have belonged to the first wave of immigrants carrying farming technology from south-eastern Europe or Anatolia into central Europe. DIN2 is from a nearby site, around 500 years more recent, and is thought to potentially belong to a second wave of farmers who migrated along the Danube. It is unclear to what extent these different waves corresponded to populations with different ancestries.

When painted using 465 ancient samples from the literature and the newly sequenced samples, Erg1 had the lowest *TVD* with DIN2, supporting the hypothesis that they were from the same source population. Erg1 had the second lowest *TVD* with Ess7, another LBK sample, from Essenbach, Germany. DIN2 also shares low *TVD* with Ess7, but has the lowest *TVD* with NE5, NE4 and NE7, samples assigned to Middle and Late Neolithic cultures on the Hungarian plane. DIN2 was assigned to mitochondrial haplogroup J1C, the same as the samples NE4 and NE5. Both the autosomal and mtDNA link to Neolithic Hungary supports the hypothesis that DIN2 migrated along the Danbian route.

To explicitly test whether Erg1 and DIN2 group together to the exclusion of other ancient samples and therefore, whether they likely originated from a similar source population, I performed f_4 tests in the form of $f_4(W = \text{Erg1}, X = \text{DIN2}; Y = \text{test}, Z = \text{Mbuti})$, where *test* is 143 ancient populations used in the F-statistics analysis. This tests whether Erg1 and DIN2 form a clade to the exclusion of *test* or not. Of the 143 comparisons, only the population labeled as WHG had a $|Z| > 3$, ($Z = 3.057$), suggesting that Erg1 and DIN2 originate from the same local population. However, this result was surprising given we

would not typically expect an individual from the LBK culture to form a clade with hunter-gatherer populations; this could be indicative of gene flow between a WHG-like source and Erg1. This result was robust to outgroup choice.

To determine whether Erg1 showed increased genetic similarity to local farming populations, I also performed combinations of f_3 in the form of $f_3(A = Erg1, B = test, C = Mbuti)$, where *test* iterates across 143 ancient populations. This tests the branch length, or the amount of genetic drift that has occurred on the branch between Erg1 and *test* since their divergence from an outgroup. The sample/population with the highest f_3 statistic was NE7, a sample from 4,360 – 4,490 BC and the Lengyel culture (a Neolithic culture centered on the Danube River, known to be an offshoot of the LBK culture Erg1 belonged to). On the other hand, DIN2 shows a clear affinity to samples from Neolithic France.

I obtained SNP-capture data from several other local LBK populations; samples from Schwetzingen, Stuttgart-Mullhausen and Halberstadt. These samples appear to form a distinct cluster on the plink PCA and are shifted away from the primary cluster of Neolithic individuals and towards samples from the Anatolian Bronze Age and Baden Culture (a central European Chalcolithic culture). I wanted to know which LBK population Erg1 and DIN2 were closest to. I found strong evidence ($|Z| = 7.97$) that Erg1 shared more alleles with LBK populations from Schwetzingen than with Stuttgart-Mühlhausen, suggesting the early LBK populations showed relatively fine-scale geographic structure. Given the lack of Hunter Gatherer ancestry in the Rivollat LBK samples, this structure seems unlikely to be driven by variable amounts of Hunter-Gatherer admixture (Fig. 4.7).

4.3.3 Variable amounts of local hunter-gatherer ancestry in Neolithic farmers indicates a structured population

Prior research has shown that admixture occurred between newly arrived farming immigrants from Anatolia and local hunter-gatherers [81, 89]. The position of Erg1 on the PCA suggests that it may have a significant component of Hunter-Gatherer ancestry. I applied the SOURCEFIND algorithm to the ‘ancients painting’ co-ancestry matrix to infer ancestry proportions for all newly sequenced individuals, fixing 3 surrogate populations at WHG, Yamnaya and Anatolian Neolithic (Fig. 4.8). I inferred 26% WHG ancestry in Erg1, suggesting it may have had a relatively recent ancestor who was a Hunter-Gatherer. I inferred a smaller proportion of WHG ancestry into DIN2 (8%), perhaps suggesting that they were part of a structured local population, where different elements received varying amounts of hunter-gatherer admixture. qpAdm modeling broadly agreed with these estimates and showed that Erg1 can be modeled as a mixture of Anatolia Neolithic (61%, $se=0.095$) and WHG (0.3855%, $se=0.095$). Erg2 showed no evidence of hunter-gatherer ancestry and could be modeled directly as Anatolian Neolithic farmer.

To localise the closest source of Hunter-Gatherer admixture into Erg1, I re-performed the 3-population SOURCEFIND analysis, but instead split up the WHG surrogates into Loschbour, LaBrana, Bichon and the 2 individuals from the Iron Gates, leaving 6 surrogate populations in total. I inferred that the two 8800-year-old Iron Gates individuals from Serbia contributed towards 33% of the ancestry of Erg1, showing that it was likely to be closest population to the mixing source in our dataset. To confirm that this was not an artefact of there being 2 Iron Gates individuals (where all of the other WHG populations had a single sample), I removed the lowest coverage Iron Gates individual from the surrogate pool and repeated the analysis. The proportion of ancestry inferred from Iron Gates was similar (31%), suggesting the sampling did not affect

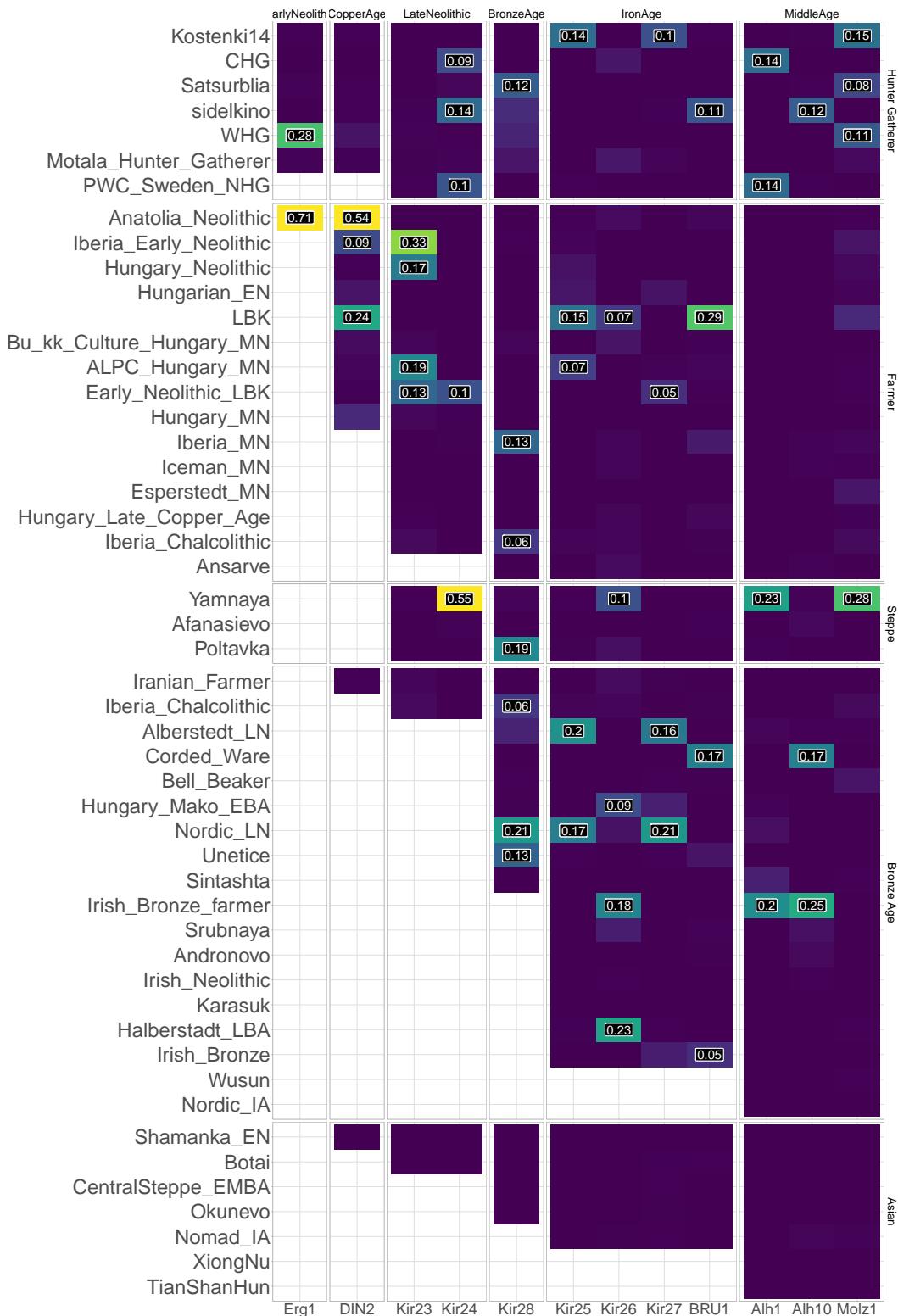


Figure 4.5: SOURCEFIND ancestry proportion estimates for all newly sequenced target samples (vertical columns). Target samples are grouped by archaeological age. Surrogate populations are represented as horizontal rows and also grouped into archaeological culture. Each target was modeled as a mixture of only populations which are dated to being older or contemporaneous as the target. Numbers within each cell correspond to the ancestry proportion estimate.

the inferred proportion. The same result occurred across all 5 independent SOURCEFIND runs.

To determine the date of admixture between an Anatolian Farmer-like and WHG-like source into Erg1, I used MOSAIC [78], which infers admixture events using a similar technique to chromosome painting. MOSAIC is able to model the ‘true’ admixing sources and determine the genetic differentiation between those and the sampled sources, in addition to the date of admixture. When modeled as a 2-way admixture event, MOSAIC inferred similar WHG and Anatolia Neolithic mixing proportions to SOURCEFIND. It inferred the cluster of Italian hunter-gatherers to be the closest population to the true mixing source (Fig. 4.6). MOSAIC is able to infer the Fst between the ‘true’ mixing groups and the sampled populations. I inferred very low Fst between the true and source populations, suggesting we had sampled a good proxy for the ‘true’ mixing sources. I inferred an admixture date of 5.3 generations before the Erg1 was alive. I caution that the admixture date may be unreliable due to only targeting a single individual and given MOSAIC bootstraps over individuals (rather than over Chromosomes as in GLOBETROTTER or LD blocks as in qpAdm), it was not possible to obtain confidence intervals around admixture date.

To confirm this admixture event, I performed an f_3 admixture test, which, when significantly negative, provides unambiguous evidence of an admixture event [13]. I performed the test $f_3(A = \text{Castelnovian_Mesolithic}, B = \text{Anatolia_Neolithic}, C = \text{Erg1})$, selecting the A and B populations as those were inferred by MOSAIC to be closest to the admixture sources. This did not yield a significant result ($Z = 1.96$). However, exchanging Anatolia_Neolithic for LBK, a source temporally and geographically more proximate to Erg1 yielded a significant result ($Z = 4.25$).

I also obtained SNP-capture data from several other local LBK populations; samples from Schwetzingen, Stuttgart-Mullhausen and Halberstadt (Rivollat

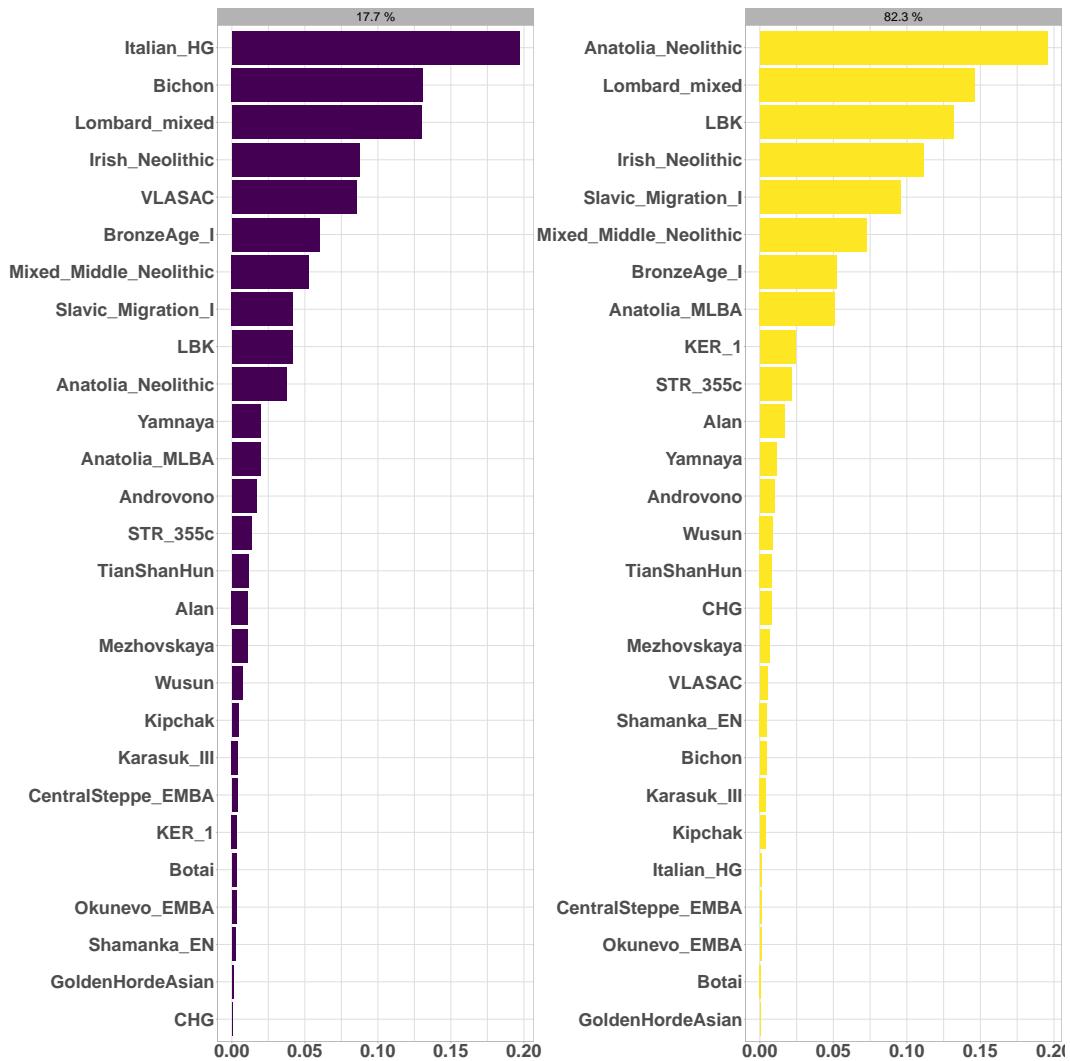


Figure 4.6: Copying matrix plot for sources in 2-way admixture event for Erg1. Each panel represents one of the 2 mixing sources. Labels above each panel give the proportion that mixing source contributed to the Early Middle Age samples. Length of the bars within each panel represent the amount that mixing source copied from a particular population.

samples). These samples appear to form a distinct cluster on the unlinked PCA and are shifted away from the primary cluster of Neolithic individuals and towards samples from the Anatolian Bronze Age and Baden Culture (a central European Chalcolithic culture) (Fig. 4.3). I wanted to contextualise the amount of Hunter-Gatherer in the newly sequenced samples, compared to different French and German farmer groups. As expected, and shown by previous studies [90], Early Neolithic populations show little sign of Hunter-Gatherer ancestry, which appears more into the Middle Neolithic and further west from Greece and Anatolia. Populations from France, in particular early samples, show the highest amount of HG ancestry. However, contemporaneous populations from Germany display much reduced levels of HG ancestry and can fit a model of purely Anatolia Neolithic ancestry well. Our sample Erg1 appears to be an exception, displaying high levels of HG ancestry comparable to the samples found in France (Fig. 4.7). On the other hand, Erg2, a sample which is contemporaneous and local to Erg1, showed no evidence of Hunter Gatherer admixture

4.3.4 Spatially and temporally close samples in Late Neolithic display highly distinct ancestries

This dataset included 2 individuals found in the same stratigraphical layer of Cherry-Tree cave; Kir23 and Kir24 were both dated to the Late Neolithic (approx 4700 BP). Despite their temporal and spatial closeness, they show highly different ancestry profiles (Fig. 4.8).

On both the plink and ChromoPainter PCA and fineSTRUCTURE clustering, Kir24 clusters with individuals from populations present around the Eurasian Steppe during the Bronze-Age, such as those from the Yamnaya and Afanasievo cultures. These are the populations known to be responsible for the spread of Indo-European languages across Europe [40]. These results support the findings of Allentoft (2015), who concluded that the Afanasievo

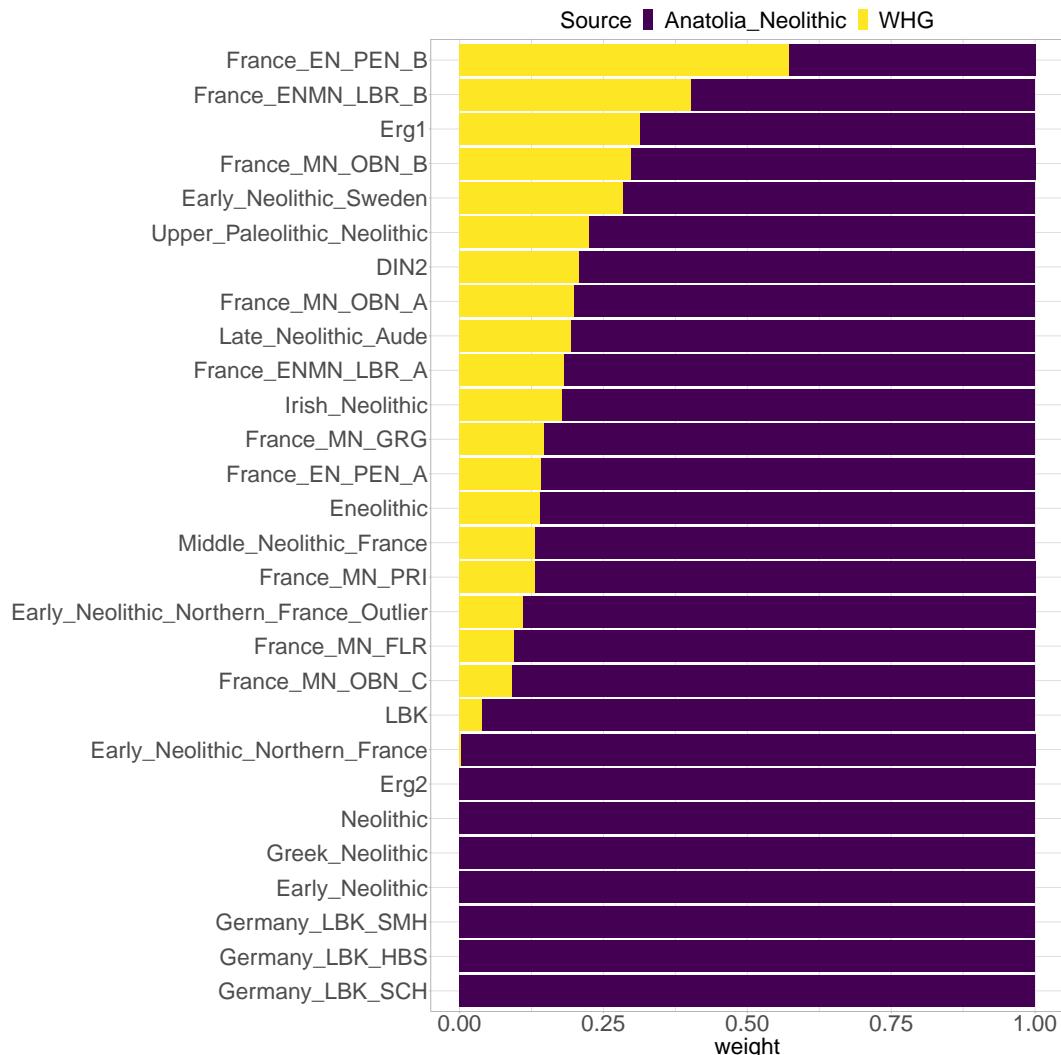


Figure 4.7: qpAdm ancestry proportion estimates for a selection of European Neolithic individuals. All individuals were modeled as a 2-way mixture between Anatolian Neolithic farmers and Western-Hunter Gatherers (WHD). Outgroups given in methods 4.2.9.

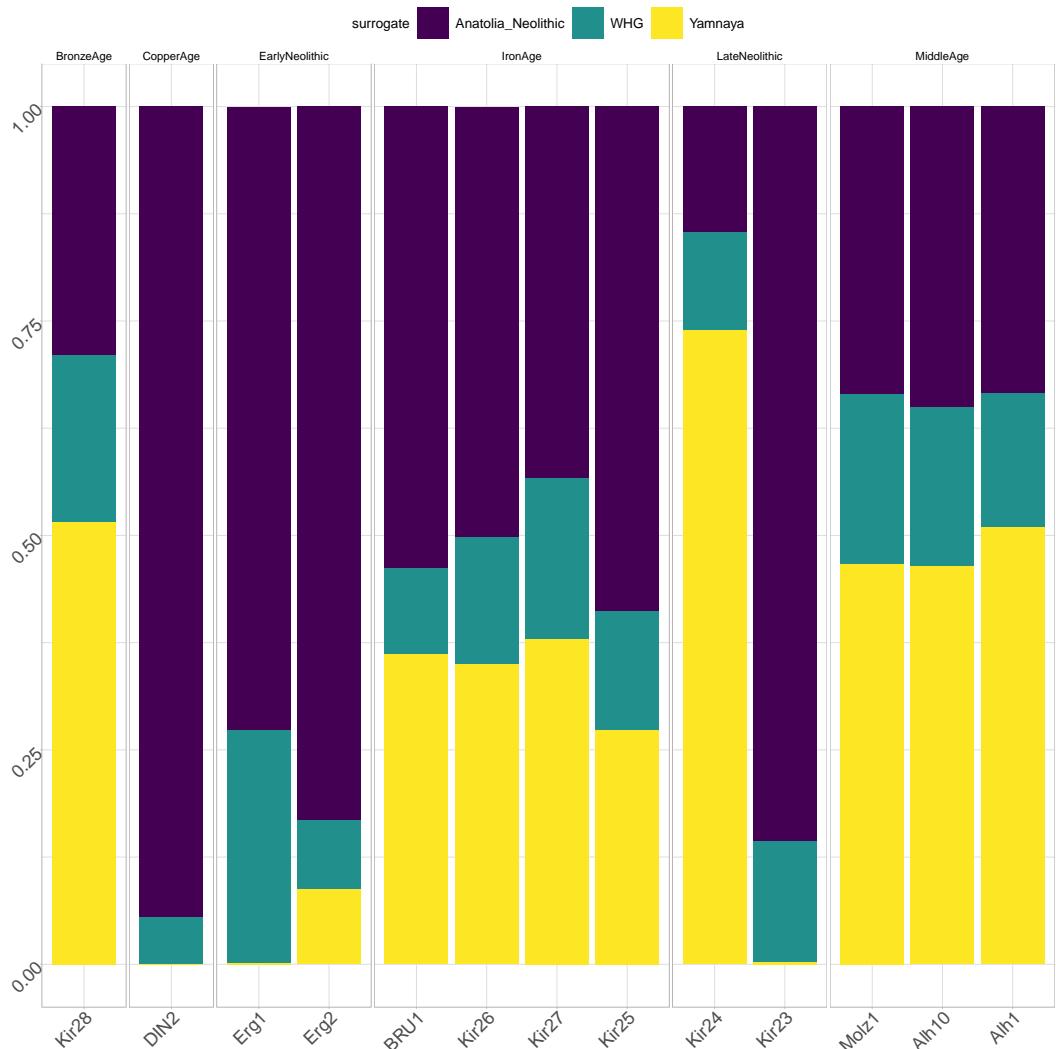


Figure 4.8: SOURCEFIND ancestry proportion estimates for all newly sequenced target samples (vertical columns). Target samples are grouped by archaeological age. Surrogate populations are represented as horizontal rows and also grouped into archaeological culture. Each target was modeled as a mixture of only populations which are dated to being older or contemporaneous as the the target. Numbers within each cell correspond to the ancestry proportion estimate.

Culture were ‘genetically indistinguishable’ from the Yamnaya Culture. That the Yamnaya and Afanasievo samples were sampled in Russia suggests that Kir24 may have been a recent migrant from the Eurasian Steppe. This is supported by IBD analysis; of all the ancient samples in the dataset Kir24 shares the most IBD (31.12cM) with Yamnaya and the lowest *TVD* with 2 other members of the Yamnaya population. This timing (Kir24 is dated to approximately 4700 BP) corresponds to some of the earliest appearance of Yamnaya-like ancestry in central Europe [91]. Using qpAdm, Kir24 could be modeled as a mixture of Yamnaya (93%, se=12%) and WHG (6%, se=8%) without any Neolithic ancestry.

Kir24 was assigned to mtDNA haplogroup T1a1, which has been found in Yamnaya samples from the Middle Volga region and Bulgaria [92]. Additionally, they found the frequency of T1a1 to be higher in the Yamnaya peoples than in any other ancient or modern population.

On the other hand, Kir23 is found in a fineSTRUCTURE cluster with Ballynahatty, from Neolithic Ireland (3343-3020 BC), and is positioned on both plink and ChromoPainter PCAs with other late Neolithic samples. It is found in adjacent fineSTRUCTURE groups to samples from Neolithic Spain and Ireland. As is the case with other Neolithic samples of this era, Kir23 has a component of Hunter-Gatherer ancestry; it is known that Middle Neolithic individuals are characterised by admixture with the existing Hunter-Gatherer populations. qpAdm modeling showed that Kir23 could be formed from a mixture of Neolithic Anatolia (96%, se=14) and Hunter Gatherer (6.25, se=0.91) without the need for additional Steppe ancestry.

To test whether the source of Neolithic ancestry in Kir23 was most similar to local populations, I performed f_4 tests in the form $f_4(W = \text{Kir23}, X = \text{mbutipygy}; Y = \text{test}, Z = \text{Erg2})$, which tests whether Kir23 forms a clade with Erg2, a local farmer individual, or *test*, where *test* was one of several different farmer populations. Erg2 was chosen as the local group because it lacked any

potentially confounding Hunter Gather ancestry. Kir23 always formed a clade with Erg2, suggesting that the source of ancestry into Kir23 was local and that there was a degree of continuity within the region.

4.3.5 Introduction of ‘southern’ ancestry to Cherry-Tree Cave during the Iron Age

Both the plink and ChromoPainter PCAs show that the Iron Age samples appear to be shifted towards the cluster of Neolithic individuals relative to the Bronze Age. The same pattern is also seen in the modern PCA, where the Iron Age samples are shifted substantially towards Spain / Northern Italy relative to the preceding Bronze Age sample which is situated among Northern / Western European populations (Germany, Wales) (Fig. 5.7). Previous studies into the Bronze-Iron Age transition in Western-Europe (France) have shown relative continuity [93]. Other studies in Eastern-Central Europe (Hungary) have shown the Bronze-Iron Age transition was accompanied by an increase in Eastern-European ancestry (albeit from a single sample) [89]. I was interested to see whether the transition in Bavaria had elements of either of these phenomena.

To identify the possible source of ‘southern’ ancestry in the Iron Age samples, I formed each of the Bronze Age, Iron Age and Middle Age Bavarian populations as a mixture of all other ancient populations using SOURCEFIND. I detected a component represented by ‘Renaissance’, a population from approximately 1500CE Italy, which contributed towards 26% of the ancestry to Iron Age individuals, but was found in neither the preceding Bronze Age nor following Middle Age. Thus, Renaissance samples appear to be the closest proxy for the ‘southern’ ancestry source. qpAdm modeling showed that the Iron Age samples can be well formed from a mixture of the preceding Bavarian Bronze age sample and those from either Renaissance Italy, Imperial Rome, Imperial Rome Late Antiquity or ‘Roman Solider’ from Veeramah et al (2018). All other possible sources included with Bronze Age resulted into poorly fitting models.

This suggests a model of admixture from populations best represented by those from post Iron-Age Italy.

To determine whether this was an admixture event, I grouped the Iron Age samples together and performed MOSAIC admixture analysis. In the 2-way admixture model, the Iron Age samples could be formed of a mixture of a source closest to an Alamannic-Frankish sample (510 – 530 AD) 17.7% and a source closest to Anatolian Neolithic / LBK samples (82.3%). The estimated F_{st} between the 2 mixing sources was 0.016, approximately equivalent between present-day Germans and Palestinians [94]. Bootstrapped dates estimated the date to between 7.86 and 11.31 (95% quantiles) generations ago. This signal is supported by the fineSTRUCTURE groupings; all 4 Iron Age individuals were grouped alongside several Lombard samples and a Roman solider from 300AD.

Based on SOURCEFIND modeling with the extended older surrogates set, unlike Gamba et al (2014) [89], I found no evidence of East-Asian or East-Asian-like admixture (Fig. 4.5).

4.3.6 Present-day genomes unpick genetic differences between early Germanic and Slavic populations

Finally, our dataset included 3 samples from the Middle Age period. The two genomes from Altheim, Germany, date to around 500AD and were found in a Roman context. The single individual from Molzbichl, Austria, dates to around 300 years later, and has been assigned to a ‘Slavic’ context.

The 3 Middle Age samples appear to share common ancestry based on the plink PCA and are located next to other samples from the Middle Ages. Some structure is apparent from the ChromoPainter PCA, with the two Altheim samples clustering more closely together to the exclusion of the Slavic sample; however, this difference appears to be subtle. f_4 in the form $f_4(mbutipygmy, Bavaria_Iron; Bavaria_Slav, Bavaria_Germanic)$

returned a non-significant result, showing that samples from the Iron Age in Bavaria were symmetrically related to the later Middle Age sample. These results suggest that the differentiation between ‘Germanic’ and ‘Slavic’ populations arose post Iron Age. However this non-significant result could be caused by low sample sizes in the Middle Age populations or a lack of power in allele-frequency based methods.

The two Germanic samples fall into a fineSTRUCTURE cluster with a set of contemporaneous samples from Northern Europe, including 10-11th century Vikings from Estonia, Sweden and Iceland. On the other hand, Molz1 clusters with other individuals known to be from Early Slavic populations. Interestingly, the Slavic cluster also containing a sample DA29, also known as ‘GoldenHordeEuro’. This sample is from Karasuyr, Kazakhstan, and was dated to 1200-1400 CE. The Golden Horde was a Mongol khanate established in the 13th Century CE. Given this sample shows clear evidence of European ancestry and clusters alongside individuals from Early Middle Age Europe, it has been proposed that this individual was captured in Europe during the Mongol raids of the 13th Century, when they assaulted the Kievan Rus’ federation. That ‘GoldenHordeEuro’ clusters with Molz1 suggests the location of capture in Europe may have been from Austria where Molz1 was found.

It is currently unknown whether, in addition to cultural and linguistic differences, genetic differentiation exists between the ‘Germanic’ peoples represented by the two Altheim samples, and the ‘Slavic’ peoples represented by the Molzbichl sample. All 3 samples are positioned close on the ancients PCA, suggesting they lack differentiation in the context of ancient samples. However, their positions on the modern PCA reveals there was strong differentiation between early Slavic and Germanic peoples (Fig. 5.7). Molz1 clusters with present-day Slavic speaking populations such as Poland, Ukraine and Belarus. On the other hand, the two Germanic samples cluster with present-day individuals from Germanic-speaking countries in Western Europe, such as Scotland,

Germany and Wales.

Plotting differential haplotype sharing between the Slavic and Germanic sample makes this pattern clear (Fig 4.9). There is a clear division down the centre of Europe, dividing it into East and West that shows the structure in present-day Europeans has existed since at least the Early Middle Ages.

These results were recapitulated using SOURCEFIND, where we modeled each individual as a mixture of different modern-day populations. The two samples from Altheim derived a large proportion of their ancestry to modern day Germans (81.8%, se=12.8), whereas the Molzbichl sample derived a large proportion of its ancestry from modern day Polish (77.85%, se=20.3) and Croatians (11.7%, se=9.1).

4.3.7 Sample heterozygosity and homozygosity

I calculated per-sample heterozygosity and runs-of-homozygosity for all samples above 3x coverage.

4.3.8 Discussion

I found that there was structure even within samples which were extremely spatially and temporally close. For example, Erg1 and Erg2 were found in the same layer and in the same location; yet Erg1 shows evidence of recent Hunter-Gatherer ancestry, whereas Erg2 shows no evidence of admixture. This raises the possibility that admixture between farmers and Hunter-Gatherers occurred on an extremely fine geographic and temporal scale. Similarly, the two Late Neolithic samples showed differences in genetic ancestry, with one sample possibly being a recent migrant from the Eurasian Steppe, displaying ancestry typical of the Yamnaya steppe-pastoralists and the other being of primarily farmer ancestry. These results clearly demonstrate that individuals who were likely genetically and phenotypically distinct lived amongst one another during

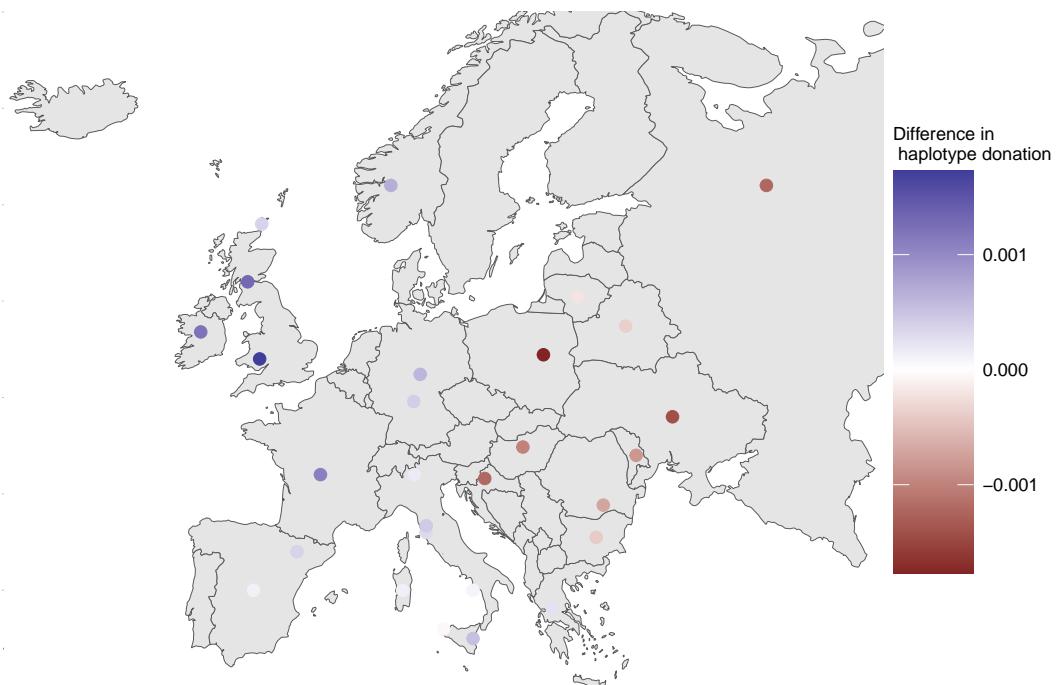


Figure 4.9: Differential haplotype-donation between Germanic and Slavic samples. Each coloured point is one present-day population. Points are coloured based on whether they donate relatively more to Germanic (blue) or Slavic (red) ancient samples.

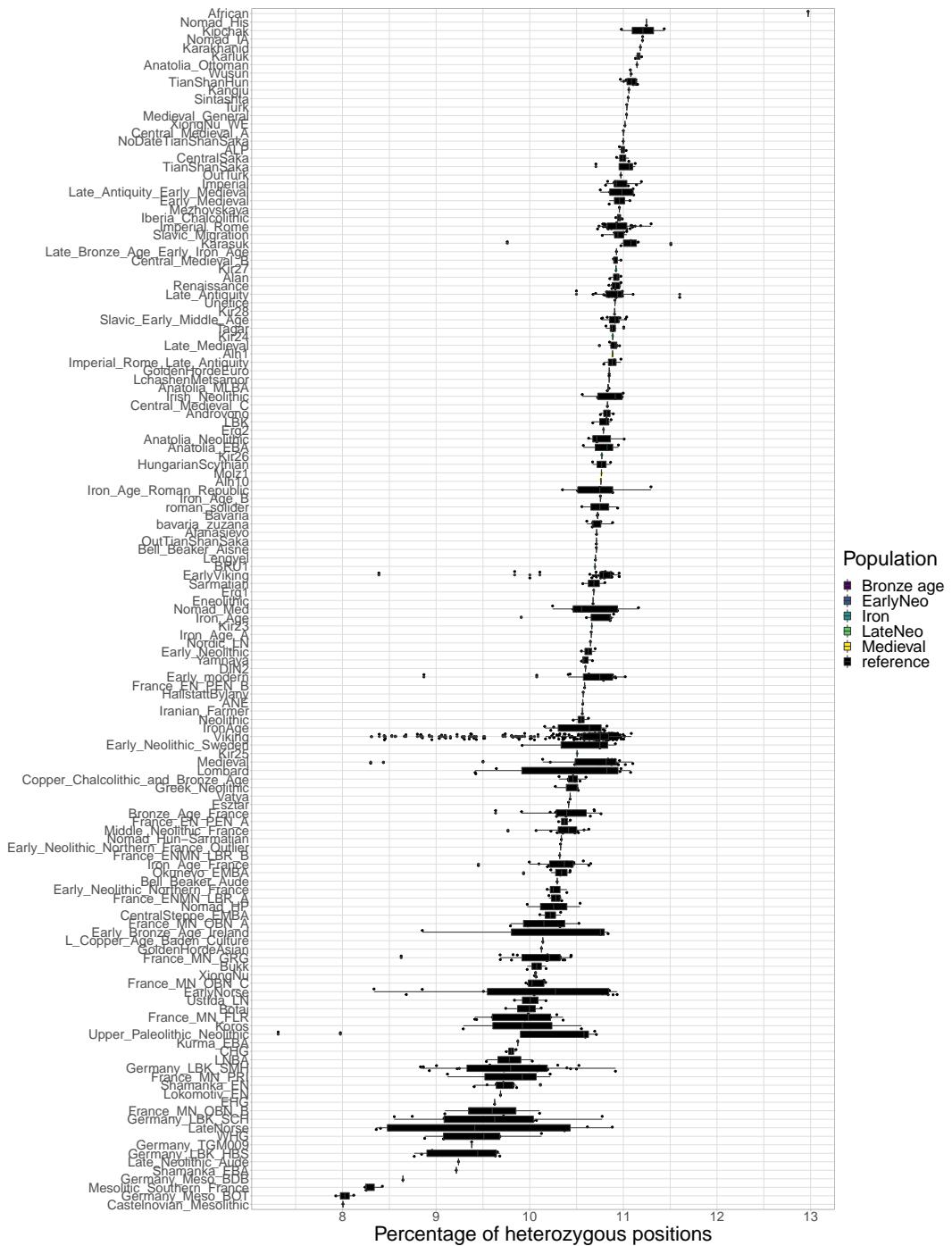


Figure 4.10

the Late Neolithic.

I found that across the different archaeological periods, within Cherry-Tree Cave, there was a degree of continuity, but with evidence of admixture from the outside.

Using 3 ancient genomes, I showed that the distinction between ‘Germanic’ and ‘Slavic’ peoples can be outlined in the context of modern samples.

Chapter 5

The genomics of the Slavic migration period, Early Middle Ages and their links to the present day

5.1 Introduction

The Slavic peoples originated as a diverse network of tribal societies who lived in Central and Eastern Europe from the first Millennia AD [95] and whose origin, although disputed, is thought to be Polesia (a marshy forested area straddling Poland, Belarus, Russiana and Ukraine) [96]. Although various Roman and Greek sources refer to Slavs as *Veneti* and *Spori* as early as the 1st and 2nd centuries AD, the term ‘Slavs’ was first used in writing by Roman bureaucrat Jordanes at the beginning of the 6th century after their attack on the Byzantine empire [97]. This era, known by historians as The Migration Period, was a period of European history, roughly between 375-568 AD after the fall of the Roman Empire [98], characterised by the large-scale movement of various peoples. The Migration Period began with the Huns moving into

Eastern Europe at the end of the 4th Century, occupying an area including present-day Hungary and Romania. During the 5th century, various Germanic groups invaded and established a homeland across parts of the Western Roman Empire. This was followed by the expansion of Slavic populations into regions of low population density in the sixth century.

Across the next 2 centuries, these peoples had settled across large parts of Europe. In particular, the Early Slavs had expanded southwards into the Balkans and Alps [95, 99–101]. It has been proposed that these migrations were key to forming the foundations of present-day Slavic (speaking) nations [95].

By the beginning of the 12th century, Slavs constituted a large part of a number of many medieval Christian states across Europe. As from this time period, Slavs could be broadly split up in 3 groups: the Eastern Slavs as part of the Kievan Rus', Southern Slavs in the Bulgarian Empire, the Principality of Serbia, Kingdom of Croatia and the Banate of Bosnia, and Western Slavs in the Principality of Nitra, Great Moravia, the duchy of Bohemia and the Kingdom and Poland. In addition, Slavic settlement also occurred in the Eastern Alps; Slovenia, large parts of present-day Austria and Friuli.

The differentiation of Slavs into these 3 broad groups can still be seen today in the different language groups. Today 315 million people speak Slavic languages. Linguistic evidence suggests that they can be broadly split into 3 groups; Western Slavs (Poles, Czechs and Slovaks), Eastern Slavs (Ukrainians, Belarusians and Russians) and Southern Slavs (Croatians, Bulgarians, Slovenians, Bosnians, Macedonians, Montenegrins and Serbians) [102].

The history of the Slavic peoples can be artificially be split into 3 periods; Migration Period (~375AD - ~568AD), Early Middle Ages/High Middle Ages (~600AD - ~1250AD) and present-day. Although previous studies have investigated the genetics of the transitions between these periods, they have been relatively limited in their scope. Juras et al (2014) used uniparental



Figure 5.1: Principle component plot of newly sequenced ancient samples and reference ancient individuals performed using the plink2. Green labels correspond to Migration Era samples, red labels to Early Middle Age samples and black as reference populations. The position of each reference label is the mean PC coordinates of all individuals within that population

mtDNA markers from ancient DNA samples from Poland to show continuity between both Roman Iron Age period (200 BC – 500 AD) and Medieval Age (1000–1400AD) with present-day Poles, Czechs and Slovaks [103]. Whilst informative about sex-biased migrations, uniparental markers carry only a fraction of the information that autosomal markers do, and therefore may provide misleading or incomplete information about the relationship between present-day and ancient samples [104] (although see [105]). For example, it is known that mtDNA and nuclear DNA may have different evolutionary histories and thus display discordant phylogenetic trees [106].

Kushniarevich et al (2015) [107] combined results from mtDNA, non-recombining Y and autosomal DNA to investigate the population structure of a wide range of present-day Balto-Slavic populations in order to understand the historical processes that have formed the present-day genetic structure. They proposed that admixture of incoming Slavic speakers during the Migration Period with the pre-existing substrate of regional genetic components, which differed between South, East and West Slavs. Using this evidence, they propose that the “cultural assimilation of indigenous populations by bearers of Slavic languages as a major mechanism of the spread of Slavic languages to the Balkan Peninsula”.

More recently, Macháček et al (2021) [108] analysed ancient rune inscriptions on a cattle rib from Lány, Czechia, dated to approximately 600AD. The bone is inscribed with Germanic runes. Finding Germanic runes in the context of Slavic peoples provides evidence of early interactions between Slavic and Germanic peoples. The bone was found in a location where Slavs were thought to have arrived at the end of the Migration Period, after the Germanic tribes had disappeared and the use of a Slavic language is historically confirmed as of the 9th century. However, whether there was early genetic contact as well is yet to be determined.

Several studies into present-day Slavic populations have detected signatures

of admixture from East-Asia [10, 78, 109–111]. Whether or not these signals can be observed in ancient individuals is yet to be seen and could further refine the admixture date. For example, different admixture dates in different Slavic populations may reveal structure among present-day Slavs.

Finally, several studies have used haplotype-based methods to explore the structure of present-day Slavic populations. Ralph and Coop [112] compared regions of IBD matching across different European populations. They found a relatively high degree of IBD sharing among pairs of individuals from Eastern Europe, suggestive of expansion from a smaller, common source population. This expansion was tentatively estimated to between 0-1000AD. Consistent with estimates of a small population size, Hellenthal et al (2014) [10] inferred an excess of IBD-sharing among Eastern European individuals, albeit with a more constrained admixture date of 440 - 1080 CE. However, this could also be interpreted in terms of a small effective population size [113, 114]. Salter-Townshend and Myers (2019) also identified admixture in the Chuvash people between East Europeans and East Asians approximately 1224 CE.

Despite these efforts, no studies have integrated autosomal DNA from ancient and present-day samples whilst applying powerful haplotype-based methods to infer population structure, ancestry proportions and admixture events. Therefore in this chapter, I will analyse 17 new medium to high coverage whole ancient genomes from Czech Republic, spanning the Migration Period and Early Middle Ages. These are, to my knowledge, the first high-coverage whole ancient-genomes from Slavic speakers. I will merge the newly sequenced samples with reference data from other ancient individuals and a large reference set of relevant present-day European individuals in order to understand their ancestry in the context of both present-day and ancient samples. In particular, I am interested in considering the following questions:

1. Can we gain an understanding of the geographical origins of the Slavic

peoples from ancient DNA

2. Do the labels “Migration Period” and “Early Middle Ages” make sense from a genetic standpoint (i.e. do samples from either period cluster with another to the exclusion of the other)
3. Was there interactions between Germanic and Slavic peoples during the Early Migration Period.
4. If so, what genetic differences can be observed between these periods? Are they characterised by admixture from outside sources? If so, what are these sources and can the admixture events be dated?
5. What is the relationship between the ancient samples and present-day day Slavic populations. Are they continuous?
6. Do the different ancient Slavic samples have different affinities to different present-day Slavic language groups?

5.2 Methods

5.2.1 Description of samples

Whole-genome sequence data was generated from 17 ancient individuals. All newly sequenced samples are from Czechia and are split across two different field sites.

The newly sequenced samples are grouped into two temporal categories; 5 samples are from the Migration Period (348 AD - 504 AD) and the Líbivá site, and the other 12 samples are from the later Early Middle Ages (724 AD - 995 AD) and the are from the Pohansko site.

Apart from the age of the samples, the Migration Period and Early Middle Age samples can be differentiated by the style of pottery found in the burial grounds (Z. Hofmanová, personal communication).

Code	Site	Date (AD)	Period	Coverage
LIB5	Břeclav z Líbivá	348	Migration	7.32
LIB4	Břeclav – Líbivá	472	Migration	6.46
LIB12	Břeclav – Líbivá	475	Migration	6.75
LIB2	Břeclav – Líbivá	495	Migration	6.39
LIB3	Břeclav – Líbivá	509	Migration	5.29
LIB11	Břeclav – Líbivá	741	Migration	5.33
LIB7	Břeclav – Líbivá	830	Migration	5.64
POH11	Pohansko – Lesní školka	783	EMA	4.99
POH27	Pohansko – Jizní Předhradí	783	EMA	5.86
POH28	Pohansko – Jizní Předhradí	822	EMA	5.58
POH41	Pohansko – Lesní školka	875	EMA	5.22
POH13	Pohansko – Lesní školka	879	EMA	5.95
POH36	Pohansko – Jizní Předhradí	880	EMA	5.47
POH40	Pohansko – Lesní školka	950	EMA	5.46
POH3	Pohansko – Lesní hrúd	956	EMA	5.39
POH44	Pohansko – Pohřebiště U Kostela	NA	EMA	5.33
POH39	Pohansko – Jizní Předhradí	866	EMA	5.30

Table 5.1: Information on newly sequenced ancient samples. Date (AD) estimated from radiocarbon dating. ‘Migration’ corresponds to Migration Period and ‘EMA’ corresponds to Early Middle Ages. Coverage calculated as the mean depth across all 77,213,942 genome-wide SNPs where genotypes were called at.

5.2.2 Ancient DNA processing

I merged the 17 newly sequenced individuals with the reference data-sets A.1 to A.17 resulting in a total of 942 ancient individuals in .bcf format, with genotype likelihoods at 77,213,942 genome-wide autosomal SNPs. Data was then split into separate .bcf files for each chromosome and indexed using bcftools.

I followed the GLIMPSE [24] imputation and phasing pipeline (https://odelaneau.github.io/GLIMPSE/tutorial_b38.html) to generate genotype likelihoods and phased genotypes for each individual. For the reference panel, I used the 30x 1000 genomes dataset [35], described in appendix A.5.

5.2.3 Present-day DNA processing

I chose the MS-POBI-HellBus dataset, described in detail in appendix A.20, because it contains a high number of relevant samples from central and Eastern Europe. I removed samples from Australia, New Zealand and USA, as these samples were not from native individuals from that country.

The modern and ancient samples were phased separately. This was because GLIMPSE, which is necessary to phase the ancient samples with, is not suitable to phase the modern samples with, for two reasons. Firstly, GLIMPSE is designed to work with sequence-level density of data, and the modern samples have been genotyped on a low-density genotyping array. Secondly GLIMPSE accepts data as genotype likelihoods; these were not available for the modern samples. Therefore, the modern samples were phased using shapeit4 [31].

Appendix A.20 describes the initial filtering that was used to generate this dataset. It was then phased using shapeit4 [31] without the use of a reference panel and setting the number of conditioning haplotypes to 8. It was then converted to ChromoPainter input format using a custom R script and merged with the dataset of ancient samples described in the previous section.

5.2.4 plink PCA

To determine the broad-scale ancestry distribution of the newly sequenced individuals in the context of 915 other ancient samples, I performed PCA on the non-imputed genotypes using plink2. Performing an unlinked PCA also allows us to identify any data quality issues which are independent of phasing / haplotype-based analysis.

I retained only the 500,000 markers with the lowest amount of missingness and then LD-pruned the resulting SNPs using the settings `-maf 0.01` and `-indep-pairwise 50 5 0.2`. PCA was performed using default settings from plink2 and the first two principle components plotted.

5.2.5 Sample heterozygosity and ROH

I used plink (v1.90p) to calculate the total length (kB) of runs of homozygosity (ROH) within each sample across all ancient and present-day individuals in the combined dataset.

5.2.6 Allele-frequency based tests

I used Admixtools [13], implemented in Admixr R library [115] to employ several different f-statistics.

I converted imputed .vcf to .ped/.map format using plink. It has been shown that using imputed markers reduced reference bias relative to using pseudo-haploid markers [17]. Convertf from the Admixtools library was then used to convert .ped/.map files into Eigenstrat format suitable for use with Admixtools.

5.2.7 ChromoPainter and fineSTRUCTURE analysis

I began with a merged dataset of present-day and ancient individuals, described in sections 5.2.2 and 5.2.3 in ChromoPainter format.

I first selected all ancient samples above 2x coverage and performed an ‘all-v-all’ painting where each haplotype was compared to all other haplotypes in turn. 2x was somewhat arbitrarily chosen as a conservative threshold to reduce coverage related bias whilst still retaining a suitable number of individuals. This allows for the characterisation of the ancestry of the newly sequenced ancient samples in the context of other ancient individuals. It is also the painting that can be used to perform fineSTRUCTURE clustering and tree building on ancient samples. Hereafter referred to as ‘ancient’ painting.

I also performed an ‘all-v-all’ painting of a selected group of present-day individuals and the newly sequenced ancient individuals. The populations

retained are given in table ???. Hereafter referred to as ‘present-day painting’.

Both the ‘present-day’ and ‘ancient’ paintings were merged separately using chromocombine-0.0.4 (<https://people.maths.bris.ac.uk/~madjl/finestructure-old/chromocombine.html>).

The fineSTRUCTURE [9] clustering and tree building algorithm was applied to the chunkcounts ChromoPainter output, for both the ‘present-day’ and ‘ancient’ paintings. This algorithm assigns individuals to genetically homogeneous clusters, estimates the ‘true’ number of clusters and builds a dendrogram of genetic similarity. This is particularly useful when combining many samples from different studies, as is the case with the ‘ancients’ painting; the population label identifiers used by different studies may not be consistent with one another. Therefore, we can use fineSTRUCTURE groupings as population labels rather than external group labels.

fineSTRUCTURE (v0.0.5) was applied to the resulting chunkcounts matrices for both the ancients painting and the moderns painting. It was first run in MCMC mode using 1,000,000 burn-in MCMC iterations and 2,000,000 main MCMC iterations. It was then run in tree-building mode (-m T) using 100,000 burn-in and 100,000 main iterations.

Tree figures, co-ancestry matrix figures and principle component plots were generated using the fineSTRUCTURE R library (<https://people.maths.bris.ac.uk/~madjl/finestructure/FinestructureRcode.zip>).

5.2.8 SOURCEFIND ancestry proportion analysis

I used SOURCEFIND [11] to infer the proportions of ancestry by which each target (e.g. ancient) individual is most related to a set of surrogate populations. Each of the 47 clusters of ancient samples inferred by fineSTRUCTURE was analysed in turn, using the other 46 clusters to act as surrogates.

Population	nsamples
HB:tsi	196
HB:spanish	68
HB:bulgarian	62
HB:german	60
HB:french	56
HB:russian	50
HB:greek	40
HB:ukrainian	40
HB:croatian	38
HB:hungarian	38
HB:norwegian	36
HB:southitalian	36
HB:polish	34
HB:romanian	32
HB:mordovian	30
HB:cypriot	24
HB:northitalian	24
HB:lithuanian	20
HB:siciliane	20
HB:westsicilian	20
HB:belorussian	18
HB:tuscan	16
HB:irish	14
HB:scottish	12
HB:germanyaustralia	8
HB:welsh	8

Table 5.2: Name of population and number of samples used in the present-day ChromoPainter analysis

Each cluster was run across 3 independent MCMC runs, using 50,000 burn-in iterations, 500,000 main iterations, thinned every 5 iterations. All 3 MCMC runs were then combined to form an MCMC list using the coda R library [39] and `mcmc` function to jointly estimate ancestry proportions and empirical credible intervals for each target population.

5.2.9 MOSAIC admixture analysis

I inferred admixture events, dates and proportions using MOSAIC [78].

I performed 2 different kinds of admixture modeling. First, I performed an

‘ancient analysis’ using the 47 fineSTRUCTURE clusters of ancient samples to assign groups. I analysed each cluster in turn, using all other 46 clusters as surrogates.

I then performed a ‘present-day surrogates’ analysis using a select group of present-day populations ?? and all ancient Slavic samples. I analysed each population in turn using all other populations as surrogates.

MOSAIC was run using default settings and the following sets of populations as targets and the following sets as surrogates. I formed each target as a mixture of either 2 or 3 ancestral sources. Upper and lower quantiles for admixture dates were estimated using a bootstrap procedure.

5.3 Results

Principle Component Analysis (PCA) using plink2 showed that the Migration Period samples do not all cluster together and instead fall on a cline of similarity between a cluster of Central European Middle Age/Iron Age samples (top-left) and Neolithic samples (bottom-right) (Fig. 5.2). The Early Middle Age samples are more homogeneous, with all samples occupying the broad region containing European Iron Age samples. However, samples POH39 and POH3 display an elevated affinity to samples from Early Bronze Age Ireland.

5.3.1 Mixed ancestry of migration period Slavs

In order to reveal further structure in the ancient samples, I performed an all-v-all painting of 152 ancient samples with a coverage greater than 2x. I then applied the fineSTRUCTURE clustering algorithm to the samples in order to assign them to genetically homogeneous groups.

The migration period consisted of 5 individuals from Břeclav (Líbivá), Czech Republic, from 5 different burial sites, who had radiocarbon dates corresponding

Population	nsamples
HB:han	34
HB:bulgarian	31
HB:japanese	28
HB:sardinian	28
HB:russian	25
HB:yakut	25
HB:greek	20
HB:ukrainian	20
HB:croatian	19
HB:hungarian	19
HB:mongolian	19
HB:southitalian	18
HB:chuvash	17
HB:polish	17
HB:romanian	16
HB:buryat	15
HB:mordovian	15
HB:altaï	13
HB:tuva	13
HB:evenk	12
HB:northitalian	12
HB:cambodian	10
HB:dai	10
HB:hannchina	10
HB:lithuanian	10
HB:miao	10
HB:nganassan	10
HB:selkup	10
HB:siciliane	10
HB:tu	10
HB:tujia	10
HB:uygur	10
HB:westsicilian	10
HB:yi	10
HB:belorussian	9
HB:daur	9
HB:oroqen	9
HB:xibo	9
HB:hezhen	8
HB:naxi	8
HB:tuscan	8
HB:dolgan	7
HB:chukchi	5
HB:koryake	5
HB:yukagir	4
HB:myanmar	3
HB:burya	2
HB:ket	2
HB:malayan	1

Table 5.3: Name of populations and number of samples used in the present-day MOSAIC analysis

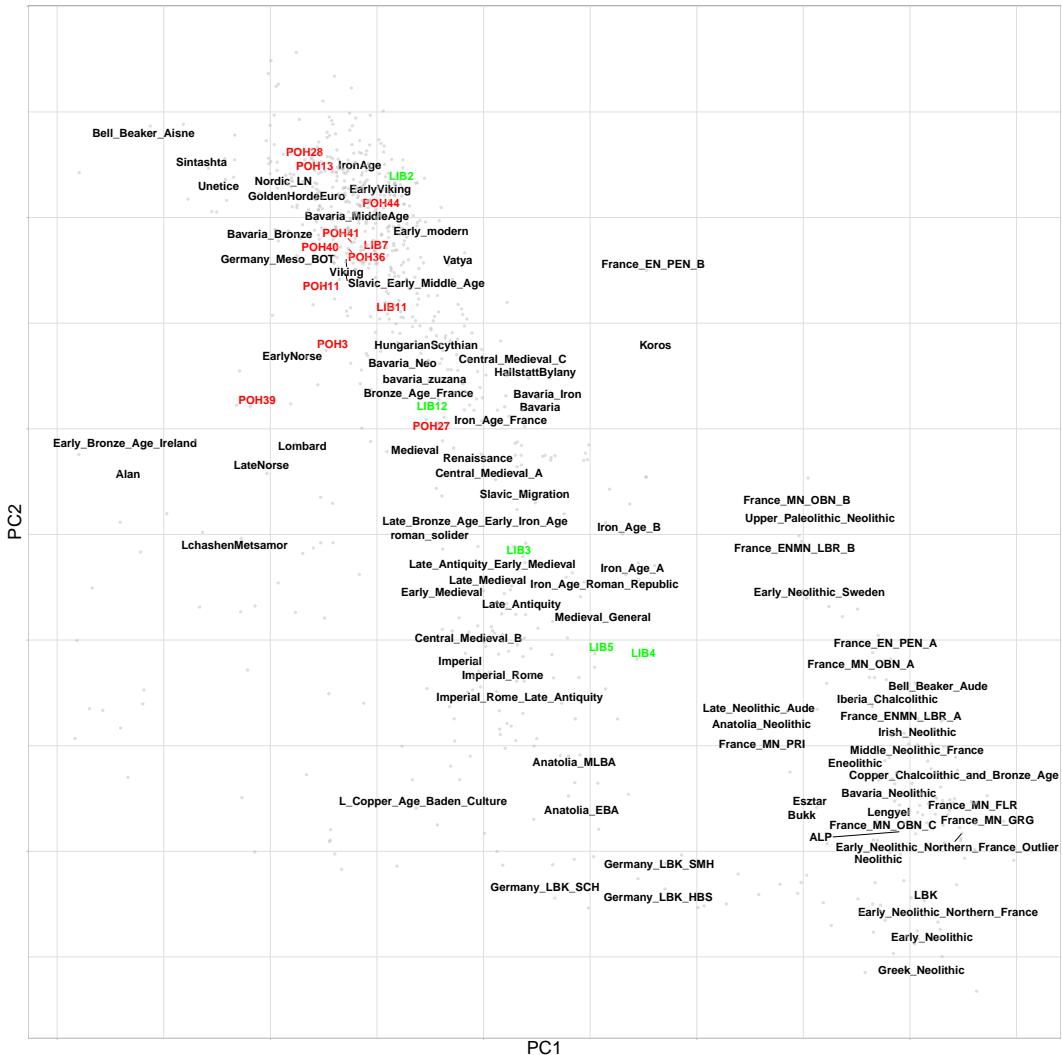


Figure 5.2: Principle component plot of newly sequenced ancient samples and reference ancient individuals performed using the plink2. Green labels correspond to Migration Era samples, red labels to Early Middle Age samples and black as reference populations. The position of each reference label is the mean PC coordinates of all individuals within that population

to the Migration Period (348 - 509AD). It is apparent from both the unlinked (Fig. 5.2) and linked PCAs (Fig. 5.3) that the Migration Period samples represent a heterogeneous group of individuals who do not originate from the same source population. LIB2 (495AD) is located in the centre of a large cluster of contemporaneous individuals from Iron Age Central and Northern Europe. This individual shares the most haplotypes with Viking individuals from Denmark, Estonia and the UK from roughly the same time period. fineSTRUCTURE analysis grouped LIB2 primarily with Viking era individuals from Sweden, Denmark, Iceland, Estonia and Norway from 300-1100AD. When painted using a set of present-day reference samples, LIB2 matches the most haplotypes and clusters with Norwegians (Fig. 5.8). Put together, these data suggests LIB2 may be a recent migrant from Viking regions.

There are many sources which detail the links between the Viking and Slavic peoples towards the end of the first millennium [116,117]. However, most evidence suggests these links occurred later than the date of these samples. For example, it is known that the Scandinavian colonists settled in present-day Russia as early as 750. Therefore, we could suggest that this is evidence of an earlier link than previously known. In their large-scale study of ancient DNA of Viking samples from across Europe, Margaryan et al (2020) present Viking samples and ancestry in Estonia, but not until the beginning of the 8th Century, some 200 years after the estimated date of LIB2.

On the other hand LIB4 and LIB5, and to a lesser extent LIB3, show an affinity the European Neolithic, indicated by their position on the linked and unlinked PCA. Interestingly, they share the most haplotypes with several Italian Neolithic samples, despite being separated by approximately 6000 years (not a clue why this is). Despite sharing the most haplotypes with these samples, LIB4 and LIB5 are found in fineSTRUCTURE clusters with more recent samples from Italy (Early Iron Age / Renaissance), suggesting the link to Neolithic Italy may have been transmitted by more recent populations (need to expand more

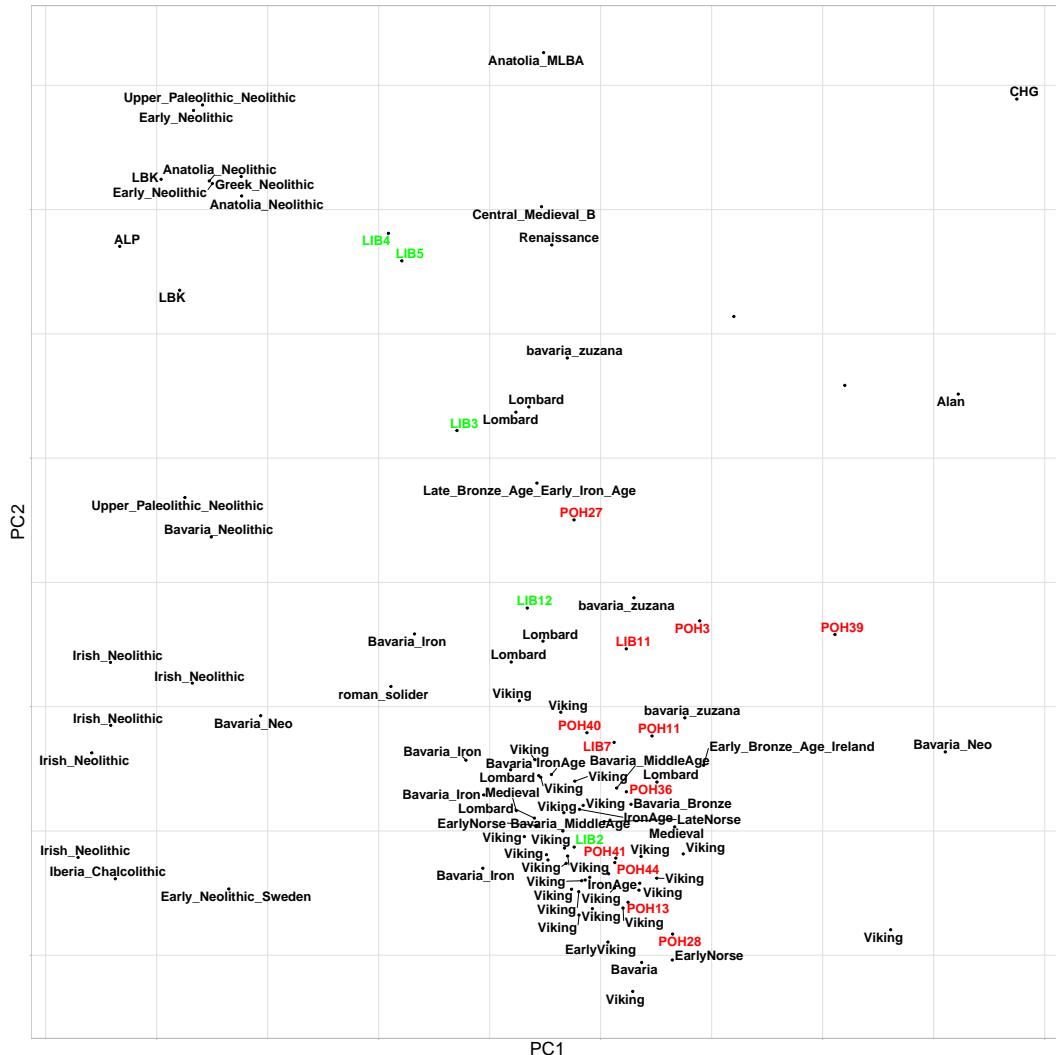


Figure 5.3: Principle component plot of newly sequenced ancient samples and reference ancient individuals performed using fineSTRUCTURE. Green labels correspond to Migration Era samples, red labels to Early Middle Age samples and black as reference populations.

on this). Both LIB4 and LIB5 share the most haplotypes with one another; this and their consistent positions on PCA and fineSTRUCTURE groupings suggest they are closely related and could be from the same local population.

The appearance of Southern-European ancestry in Central Europe, most closely related to Neolithic farmers, into the first millennium is similar to a signal found in a study exploring the ancestry of individuals with elongated skulls in medieval Bavaria (approximately 500AD) [118]. It was shown that particular individuals harbour substantial Southern-European ancestry from outside of Bavaria, closest to individuals from present-day Greece and Turkey. There are at least two possible explanations for the presence of this ancestry in the Migration Era samples. Firstly, LIB3, LIB4 and LIB5 may be similar migrants to the region. This is consistent with the fact that (at least LIB3, need to check others) is female; Veeramah et al (2018) showed that there was a tendency for females to migrate from southern regions, perhaps related to the formation of strategic alliances. Alternatively, it is possible these individuals are a leftover from a historically described Lombard migration that moved from Northern Germany (nobody really knows where it started according to Zuzana) through Czechia, Slovakia, Hungary and ended up in Lombardia. Accordingly, this could appear as genetic similarity to present-day populations from Northern Italy. This hypothesis is supported by the clustering of LIB3, LIB4 and LIB5 with present-day Italian samples in the ‘present-day’ fineSTRUCTURE analysis (Fig 5.11).

Ancestry proportion estimation using SOURCEFIND showed that the cluster containing LIB3, LIB4 and LIB5 shares 25% of their ancestry most recently with people from Anatolia, 16% from LBK (Linearbandkeramik) and 12% from a cluster containing Lombard individuals.

I performed MOSAIC admixture modeling using present-day samples as surrogates and the clusters of newly sequenced ancient samples as targets. I did not detect an admixture even when targeting LIB3, LIB4 and LIB5. This

could be due to low power or a low number of samples, or that the samples are unadmixed with respect to the surrogate populations.

On the fineSTRUCTURE PCA, LIB3 clusters with Lombard samples from Northern Italy. Historical evidence cites alliances between Slavs and Lombards in the 5th century [119]. In the ‘present-day’ painting, LIB3 clusters with and shares the most haplotypes with present-day Tuscans.

Finally, LIB12 displays ancestry which is more typical of the preceding Central European Bronze Age. It copies the most haplotypes from samples from Bronze Age Ireland (Rathlin) and Bavaria and is found in a cluster with several other Bronze Age samples. This suggests it may represent a ‘leftover’ from a local Bronze Age population which was unaffected by the Antiquity / Iron Age migrations to the region.

5.3.2 Early Middle Age Slavs represent a relatively homogeneous group typical of European Middle Ages

In comparison to the 5 Migration Period ancient Slavs, the 12 Early Middle Age Slavs (741-956 AD) represent a more homogeneous set of samples. All 12 samples were clustered into the same fineSTRUCTURE group (named Slavic Early Middle Age II), alongside Viking/Medieval samples from Ukraine, Poland and Sweden. SOURCEFIND analysis showed that this cluster derives roughly equal parts of ancestry from the clusters Viking 10C Scan I, BronzeAge I and Lombard mixed cluster. Interestingly, these are 3 ancestry sources which are similar to those identified by SOURCEFIND analysis in the Migration Period samples. We could tentatively therefore suggest that the Early Middle Age Slavs were formed from the mixture of ‘Northern’ (represented by Viking) and ‘Southern’ ancestries (represented by Lombards) onto a substrate of local Bronze Age populations. Note that I suggest that these are the most representative populations and not necessarily the ‘true’ populations that mixed.

MOSAIC admixture modeling using ancient surrogates proved inconclusive. However, using present-day individuals as surrogates provided cleaner results. The best fitting model was a 3-way admixture event involving sources closest to present-day North-Central Slavs (76.6%), Southern-Eastern Slavs (21.9%) and East Asians, best represented by Mongolians (1.5%) (Fig. 5.4). This admixture event was estimated to have occurred 9.4 (2.5% 5.7gens - 97.5% 17.9gens) generations before the samples (Fig. 5.6).

This admixture event is consistent with a signal inferred in both present-day Eastern European individuals [10, 78]. In previous studies, this admixture event was dated to approximately 1200CE (MOSAIC) and 438CE (GLOBE-TROTTER). Despite the differing dates, the proportion of ancestry is consistent across studies (approximately 2%), suggesting the signal is genuine. To further support the event, the proportion of ancestry from this source is consistent across 2-way and 3-way MOSAIC admixture models.

5.3.3 Do the samples cluster together - TVD permutation test

fineSTRUCTURE analysis suggested that the Migration Era and Early Middle Age samples did not originate from the same source population. To formally establish whether the Early Middle Age and Migration Period samples cluster within their respective populations, following Leslie et al 2015 [42], I performed a TVD permutation test. TVD is a distance metric which can be calculated from the chunklengths matrix and is equivalent to finding the absolute distance between two copyvectors, with larger values meaning two samples have more different ancestry profiles.

Using the ancients chunklengths matrix, I grouped the samples into Migration Period and Early Middle Age and calculated the average copyvectors C_{mp} and C_{ema} across samples within each groups. Then, I calculated the empirical

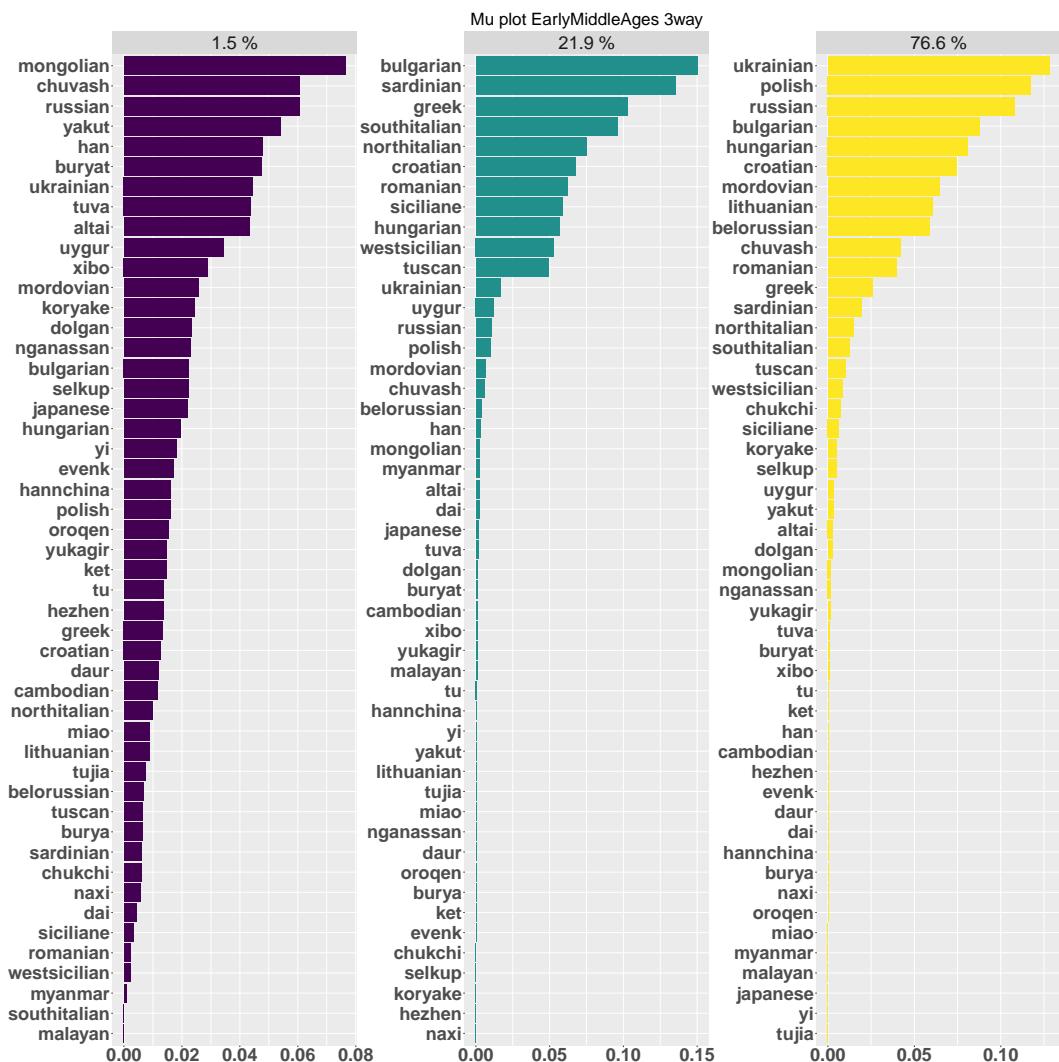


Figure 5.4: Copying matrix plot for sources in 3-way admixture event for Early Middle Age ancient Slavic samples. Each panel represents one of the 3 putative mixing sources. Labels above each panel give the proportion that mixing source contributed to the Early Middle Age samples. Length of the bars within each panel represent the amount that putative mixing source copied from a particular population.

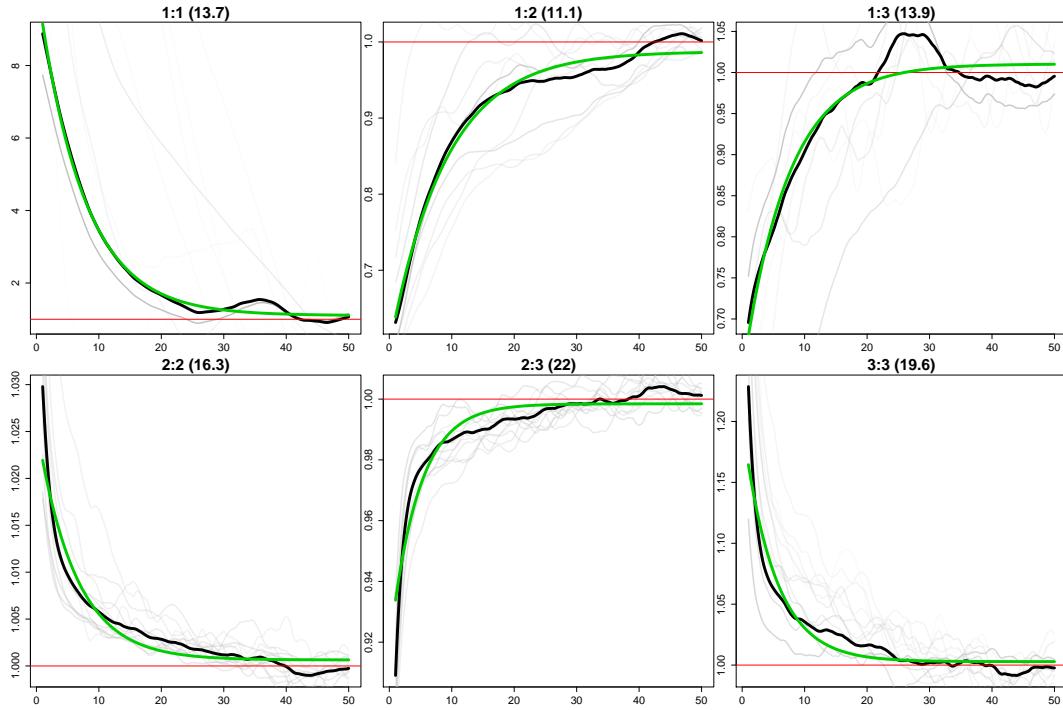


Figure 5.5: Inferred Coancestry Curves obtained from modeling Early Middle Age samples as a 3-way mixture of present-day individuals. Black lines are empirical coancestry curves across all target individuals, light grey are per individual, green is the fitted single-event coancestry curve. Note to self - need to figure out what the numbers mean but doesn't say in the manual anywhere.

TVD between the two groups as $TVD_{mp,ema} = \sum |C_{mp} - C_{ema}|$. For 10,000 iterations, I then randomly permuted the population labels among the samples and then calculated a ‘random’ TVD, $TVD_{mp,ema}^{rand}$ between the samples with randomly permuted populations. We can then calculate the p-value that we can reject the null model of no significant differences between the groups (not sure if this is the right way of wording it) as the number of randomly permuted iterations where $TVD_{mp,ema}^{rand} > TVD_{mp,ema}$. This test supported clustering the samples into their respective groups ($p = 0.0013$).

5.3.4 Interactions between the two groups

The previous section suggested that individuals from the Migration Period and Early Middle Ages had differing ancestry signals.

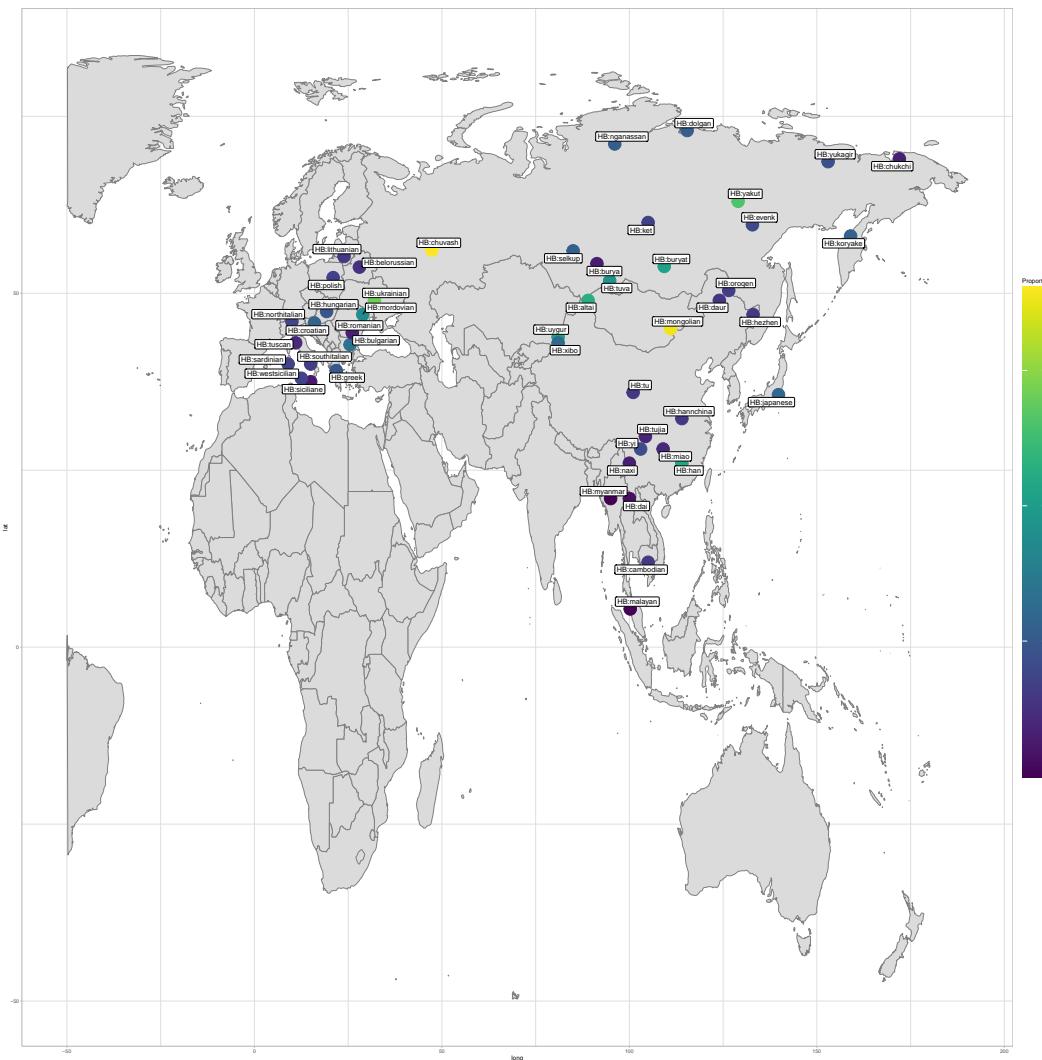


Figure 5.6: Distribution of East-Asian minor ancestry component in Early Middle Age samples.

To determine the extend of mixture and continuity between the Migration Period and Early Middle Ages, I modeled each Early Middle Ages sample as a mixture of other ancients, including individuals from the preceding Migration Period. The proportion of ancestry the individuals derive from the Migration Period clusters could be used as a proxy for the degree of continuity. The proportion of ancestry derived from the Migration Period was low (mean 3.4% , range 0.4% - 12.5%), suggesting that there was a relatively large scale population replacement between the two different time periods.

Note - I could do something like admixture f3 to see if Early Middle Age

is admixed between Middle Age and any other pop instead as a more explicit test.

5.3.5 Legacy of Slavic migrations in present-day individuals

To understand the genetic legacy the newly sequenced Slavic samples left in different European populations, I painted each sample using the HellBus dataset of present-day individuals. This dataset contains a diverse set of European populations - particularly those from present-day Slavic speaking countries (Polish, Croatian, Bulgarian, Belorussian, Ukrainian, Russian) but also neighbouring non-Slavic speaking countries (Romanian, Lithuanian, Germany and Mordovia).

Principle component analysis (PCA) of the chunklengths matrix, where present-day European samples acted as donors, reveals genetic similarity between ancient Slavic samples from the Early Middle Ages and present-day Slavic speaking people (Fig. 5.7). The samples primarily cluster with present-day Polish and Belorussian individuals, but appear to fall on a cline of genetic similarity between Russians and Southern Europeans. This cline could be mediated by the possible historical admixture event between a source closest to present-day East Asians and a second closest present-day Southern Europeans, with the position of the samples along the cline dependent on the level of admixture from the different sources.

As with previous analyses, Migration Era Slavs are spread across the PCA. 3 samples, LIB3, LIB4, and LIB5 cluster with present-day Italians, consistent with deriving a substantial ancestry component from Southern-European sources. LIB4 and LIB5 appear to be positioned closer to Southern Italians and Greeks, whereas LIB3 is closer to Northern Italian and Tuscan populations.

LIB2 shows a strong affinity to present-day Norwegians, suggesting it may

be a recent migrant from Viking regions.

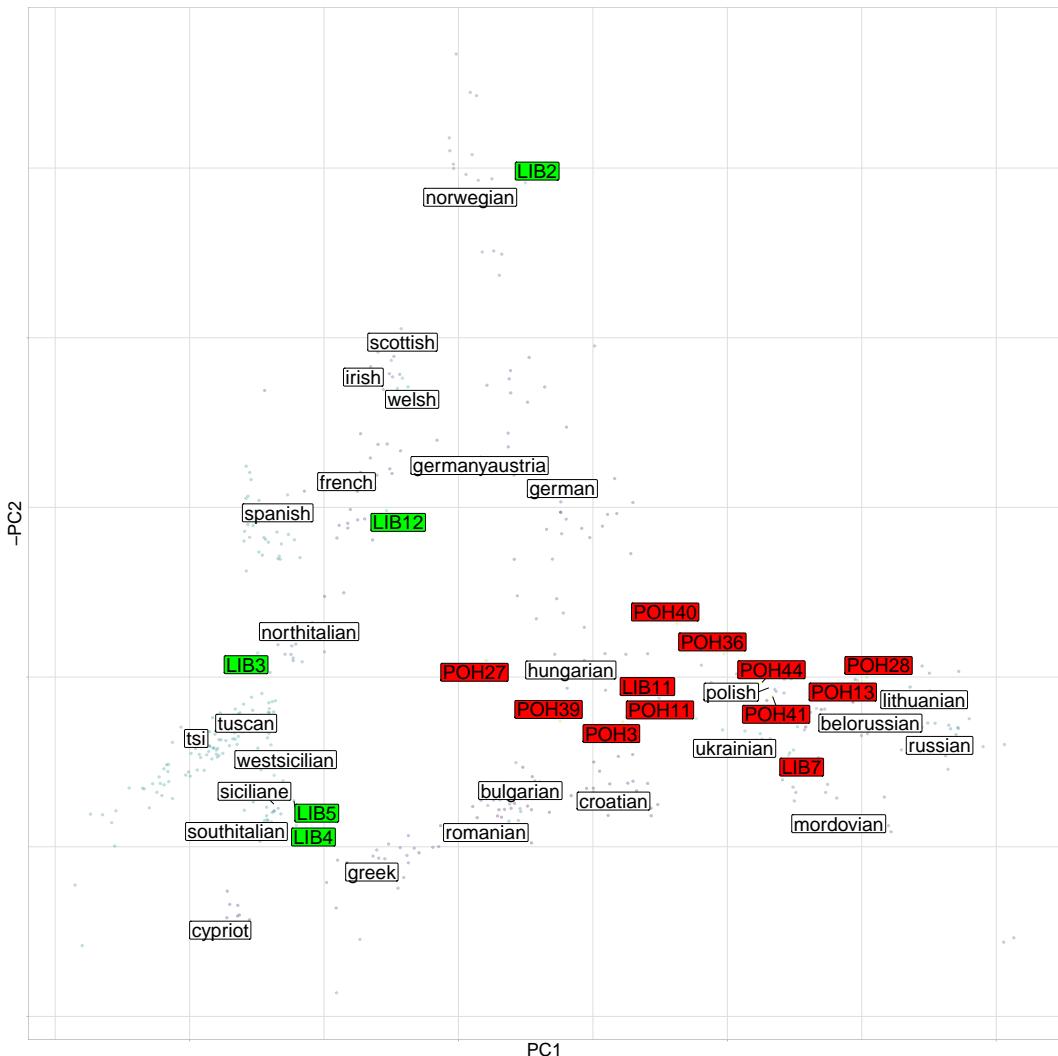


Figure 5.7: Principle component plot of newly sequenced ancient samples and reference modern individuals performed using the finestructure library. Green labels correspond to Migration Era samples, red labels correspond to Early Middle Age samples and white labels correspond to reference populations. The position of each reference label is the mean PC coordinates of all individuals within that population. Transparent coloured points correspond to present-day individuals.

The same pattern can be observed on the raw copyvector output matrix (Fig. 5.8). The Migration Era samples appear not to show any excess affinity to present-day day Slavic populations. The two samples who in previous analysis showed a strong genetic relationship to the Neolithic, LIB4 and LIB5, shared the most haplotypes with present-day day Greek individuals. This should not

be surprising given present-day day Greeks have a relatively high proportion of Neolithic ancestry relative to other European populations [120].

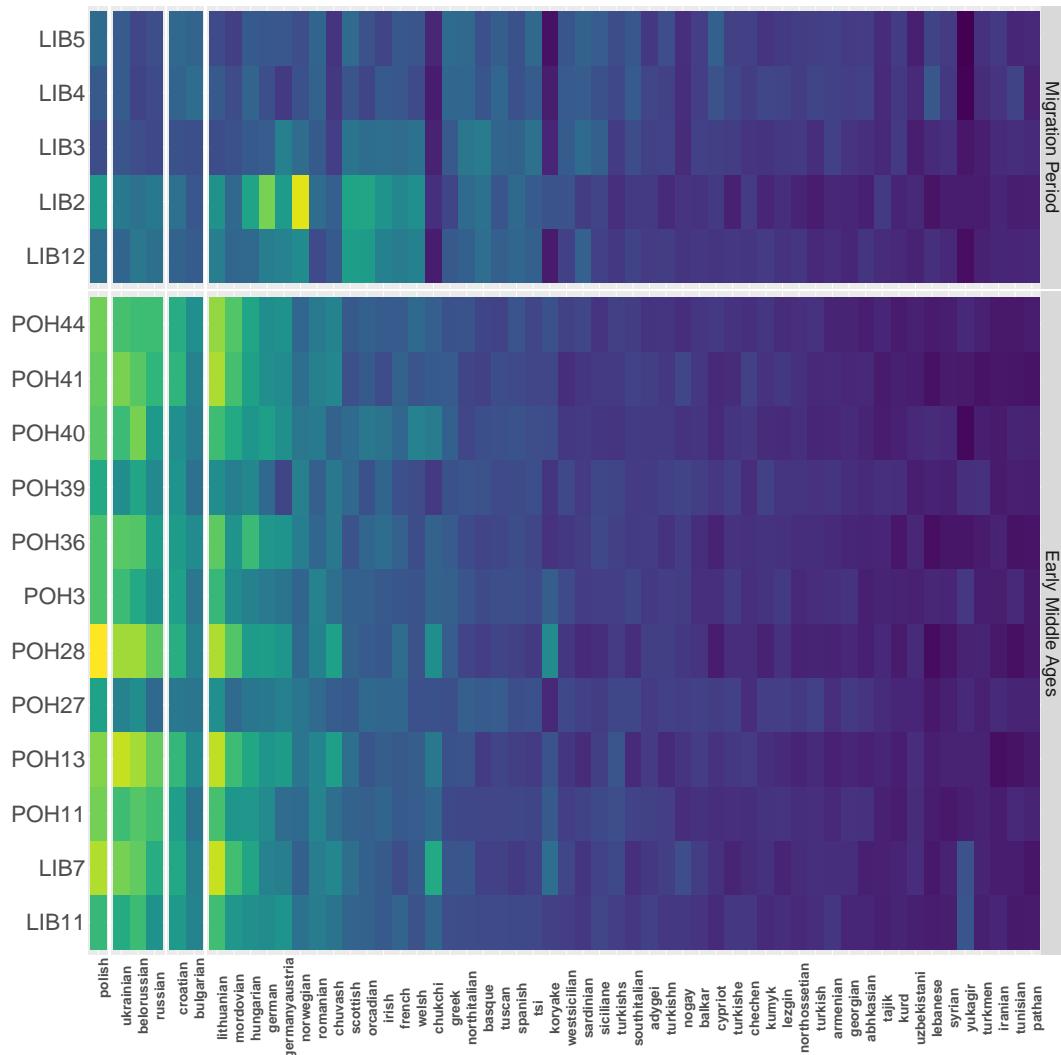


Figure 5.8: Raw chunklengths matrix from the ‘present-day’ painting. Rows correspond to different ancient recipient individuals, grouped into Migration Period and Early Middle Age period, and columns to different donor populations. Colour of cells corresponds to the total length of genome that a given donor individuals donates to that recipient, with dark/blue indicating less sharing and light/yellow colours indicating more sharing.

In contrast, the Early Middle Age samples showed a strong affinity to present-day day Slavic populations. In particular, we find that samples copy many more haplotypes from present-day day Polish individuals than they do from other populations. This is consistent with previous findings based on uniparental markers. There was also a strong affinity to several non-Slavic

speaking present-day populations - notably Lithuania and Mordovian.

To confirm that the observed results were not a result of phasing or imputing ancient individuals using present-day samples, I utilised f_3 statistics, which were performed on non-imputed genotypes. Specifically, I calculated f_3 , or the branch length / amount of shared drift, between a set of present-day test populations and the grouped Early Middle Age samples. The results are qualitatively similar to those obtained using haplotype-based methods, with Early Middle Age ancient Slavic individuals being closest to samples from Eastern Europe (Fig. 5.9). However, the f_3 results do not appear to show the same degree of geographical structure; for example, Early Middle Age have a more positive f_3 with present-day Irish individuals than with present-day Croatians.

It should be noted that f_3 statistics have the potential to be biased towards drifted groups (explain some more about this later).

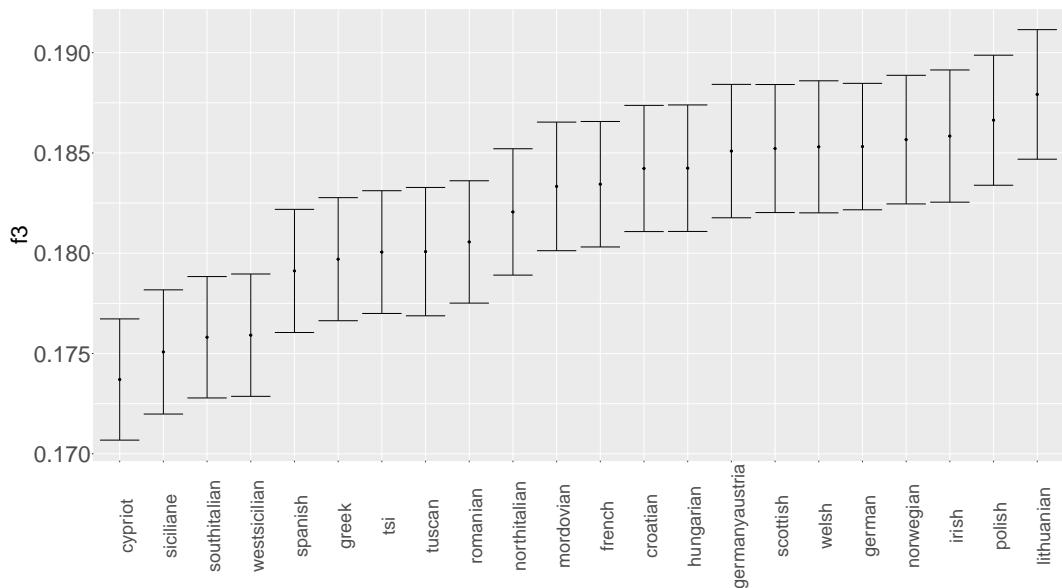


Figure 5.9: f_3 statistics in the form of $f_3(EMA, present-day; mbutipygmy)$, where *present-day* is different present-day European population. Error bars represent ± 2 standard error.

5.3.6 Continuity with present-day Slavs

The previous section strongly suggests at least some degree of continuity between Early Middle Age samples and present day Slavic populations that is not shared with the samples from the Migration Period, as the Early Middle Age samples share many more haplotypes with present-day Slavs compared to Migration Period.

To explicitly test the hypothesis that the Early Middle Age samples were continuous with the present-day Slavic populations, I used *qpWave*, which tests the number of streams of ancestry from a set of *right* populations into a set of *left* populations, $qpWave(left = croatian, lithuanian, polish, ukrainian, right = middleage, migration)$. The matrix with rank $r = 0$ can be rejected ($p = 0.112$). Note - not sure how to interpret this.

5.3.7 Genetic structure and admixture events of present-day Slavic people

As described in the introduction, several studies have investigated the structure of present-day Slavic populations, but none have integrated autosomal DNA from present-day and ancient samples and analysed them jointly with haplotype-based methods. I performed an all-v-all painting of a selection of present-day European populations and all newly sequenced ancient Slavic samples and applied the fineSTRUCTURE algorithm to the resulting chunkcounts matrix, inferring 32 clusters.

Present-day Slavs do not form a monophyletic group within the fineSTRUCTURE dendrogram to the exclusion of non-Slavic populations (Fig. 5.11), as several non-Slavic speaking populations such as German, Irish and Scottish cluster in the main clade containing Slavic speakers. Within Slavs, structure is apparent; speakers of ‘Southern’ Slavic languages from Croatia and Bulgaria form a group to the exclusion of ‘Eastern’ Slavic speaking populations from

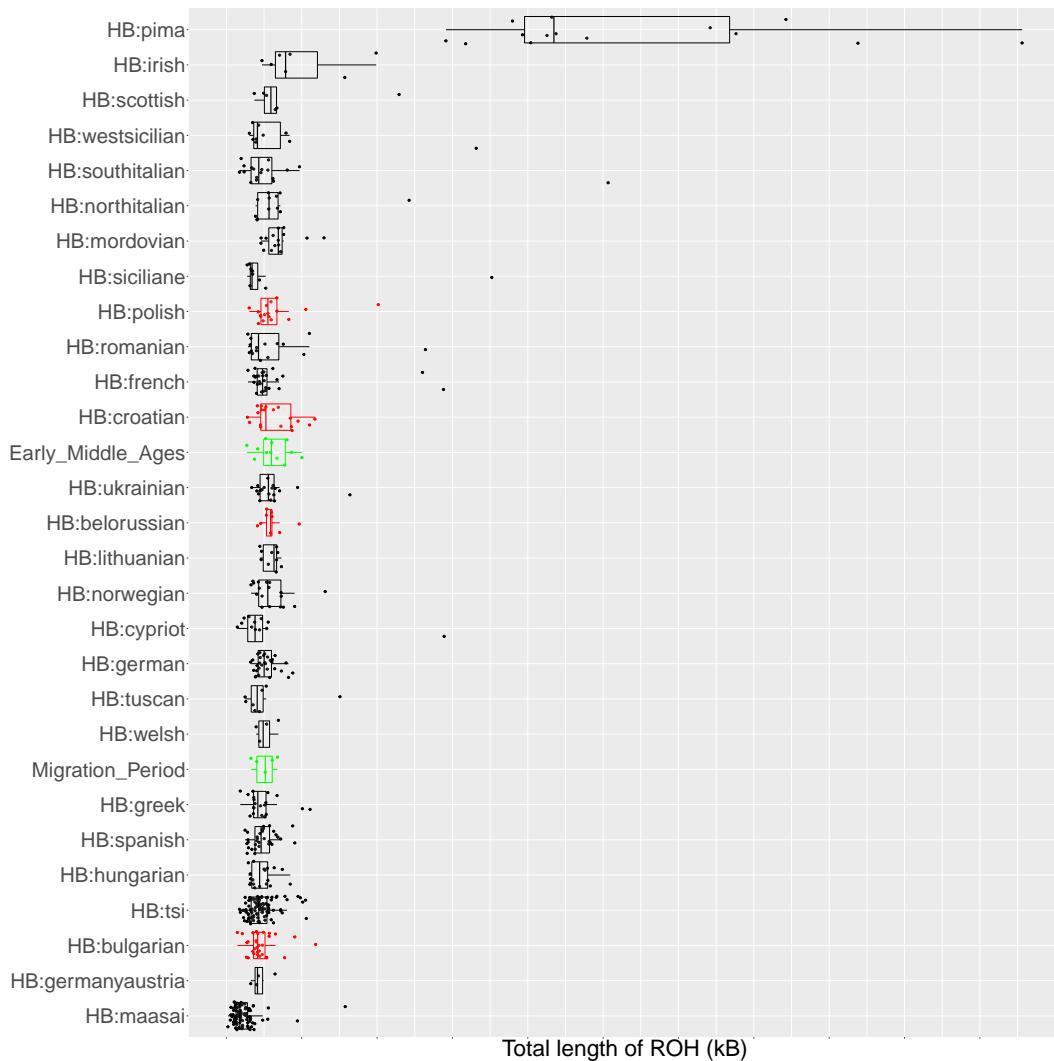


Figure 5.10: Total length of runs-of-homozygosity (ROH) in different present-day and ancient populations. Each point is the total length of ROH (kB) within an individual in that population. Points given jitter to aid visualisation. HB:pima and HB:masasai included to display extremes of ROH in different present-day human populations.

Belarus, Russia and Ukraine. Individuals from Poland cluster with ‘Eastern’ Slavic speakers, suggesting the principle axis of variation splits populations into ‘North-West’ and ‘South-East’ groups.

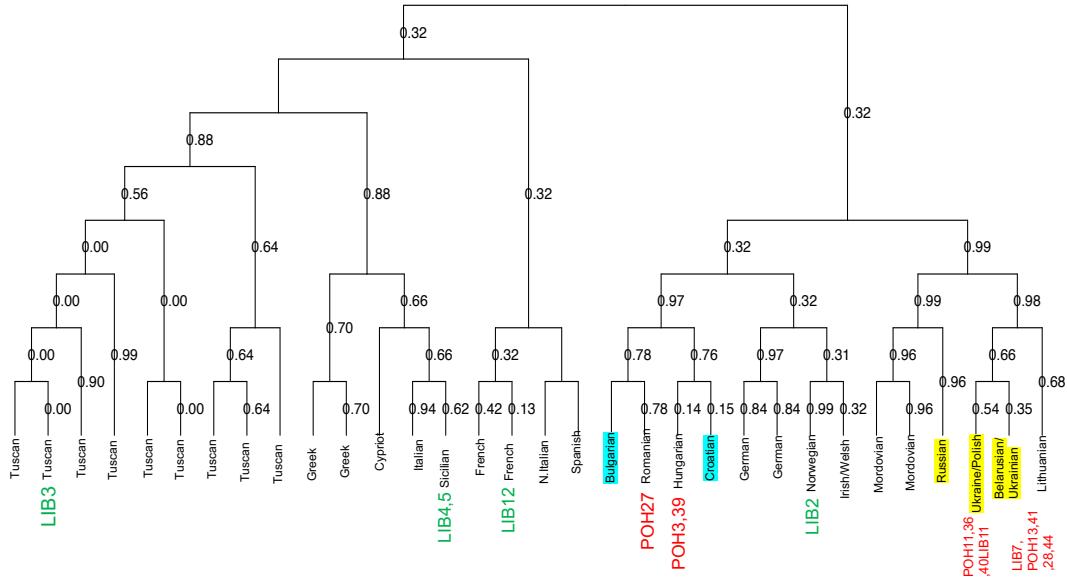


Figure 5.11: Population dendrogram generated by the fineSTRUCTURE tree building algorithm. Labeled tips refer to the primary population(s) represented in that clade. present-day non-Slavic populations shown in black. ‘South-East’ Slavs highlighted in cyan and ‘North-West’ Slavs highlighted in yellow. Migration period individuals superimposed in green and Early Middle Age samples superimposed in red. Read fineSTRUCTURE paper for description of edge values. Note: some tips contained more than one population but were not included as labels to save space.

Of the Early Middle Age samples, 3 samples (POH3, POH39, POH27) were present in the ‘South-East’ Slavic cluster, falling into a group composed of Bulgarian and Romanian samples. The remaining 7 samples are found in the ‘North-West’ cluster containing samples from Lithuania, Poland, Ukraine and Belarus. Painting the samples using present-day individuals has thus uncovered structure that was not able to be detected by looking only at ancient samples. It also suggests the structure of Slavic populations into was present at least as early as the date of these samples.

Previous studies have identified admixture events in present-day Slavic

populations involving an East Asian source approximately 440 to 1080 CE [78, 121]. In previous sections, I showed that this signal exists in the Early Middle Age ancient samples and is best characterised by populations from present-day Mongolia (Fig. 5.4).

I employed MOSAIC [78] to replicate these results and determine whether a similar admixing source is present in the ancient populations.

When considering 2-way admixture event, all of the tested populations, bar the Migration Period Slavs, showed evidence of an admixture event involving a minor source which has the lowest F_{st} with present-day Uygurs. The dates and bootstrapped confidence intervals are given in Fig. 5.12. Other than Norwegians and Croatians, whose estimated dates are later and earlier respectively, the admixture dates for other populations appear to be constrained to approximately 1250 CE. This date is similar, but slightly later than that obtained from Hellenthal et al (2014), who estimate it to be 440 to 1080 CE.

Interestingly, most present-day Slavic speaking populations, such as present-day Polish, show evidence of a 3-way admixture event, where the middle component has the lowest F_{st} with Migration Era ancient samples (Fig. 5.13). The major component has a low F_{st} with Early Middle Age Slavs. This suggests that the formation of present-day Slavic populations could have occurred via an admixture event(s) involving Migration Era individuals with high levels of Southern European ancestry, Middle Age Era samples which show a strong affinity to present day Eastern Europeans, and a small but significant East Asian source best represented by present-day Uygurs.

5.4 Discussion

The combined results from the Migration Period suggest the individuals living in Czechia during this time period were of mixed ancestry and did not originate from the same source population. The diverse set of ancestries, spanning from

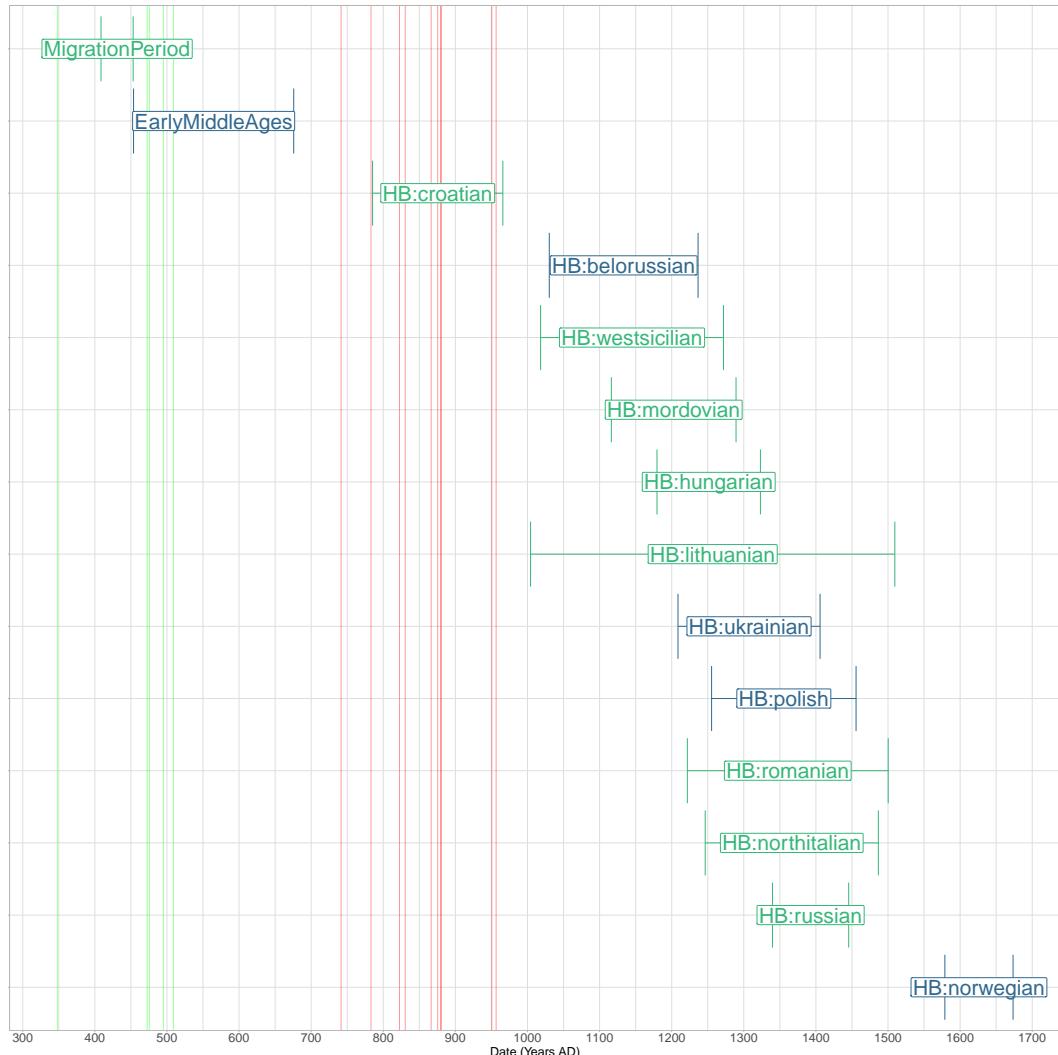


Figure 5.12: MOSAIC inferred 2-way admixture dates with bootstrapped 97.5% and 2.5% CI. Vertical green lines correspond to radiocarbon estimated dates of Migration Period samples and red lines equivalent for Early Middle Age samples. Estimated dates obtained by assuming an average generation time of 26 and date of birth of 1950 for present-day samples.

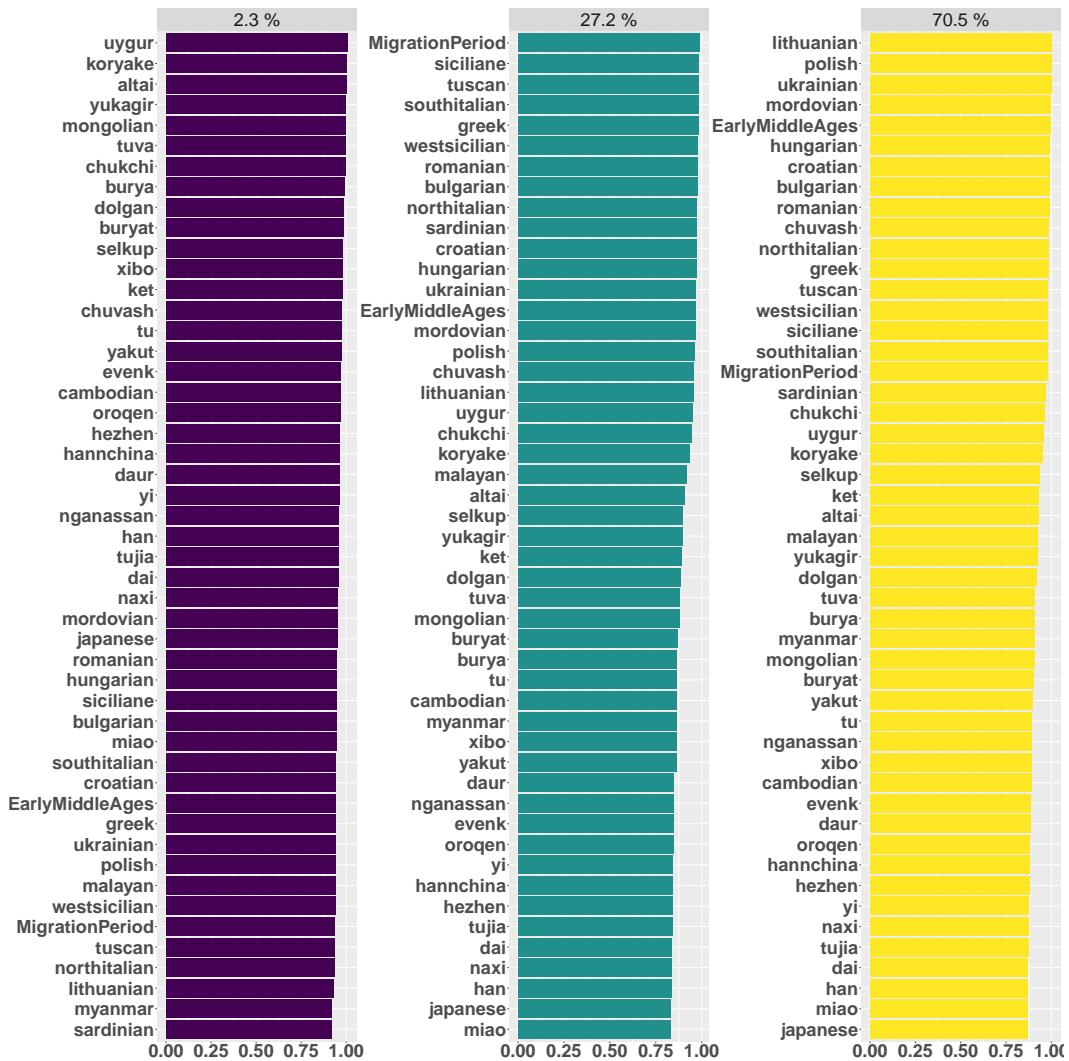


Figure 5.13: $1 - F_{st}$ between 3 inferred mixing sources for present-day Belorussians. Each panel represent a different mixing source. Each bar gives the value $1 - F_{st}$ between that samples population and the mixing source. Higher values of $1 - F_{st}$ suggest that source is well represented by a particular population.

Scandinavia to Southern Europe imply that the Migration Period was truly a period of Migration where individuals from distal ends of Europe lived among one another. In particular I inferred ancestry sources from Southern Europe and Scandinavia.

The results from the analysis of combined ancient and present-day genomes are consistent with those from Kushniarevich et al (2015) [107] who determined that Eastern (Russia, Belarus, Ukraine) and Western (Polish) central European Slavs form a cluster to the exclusion of Southern Slavs (Croatia, Bulgaria), whilst also remaining distinct from geographically proximate Germanic (German/Austrian) and Baltic (Lithuanian) populations. This is also consistent with results from Veeramah et al 2011, who showed that Sorbs, a west-Slavic population found between Poland and Germany, have a much stronger affinity to more distant Slavic populations from Czechia than to more proximate Germans [122]. Similarly, I inferred that the Slavisation of the Balkan peninsula doesn't extend beyond Croatia; the cluster of Croatian individuals only derives 1.2% of their ancestry from nearby Greek sources. However, admixture modeling suggested that Southern Slavs show signals of a historic admixture event where the minor source is related to present-day Mediterranean populations. An admixture event with a similar minor source is inferred in Migration period samples, albeit dated further in the past.

I recapitulated a previously described admixture event into not only present day Slavic speaking populations, but also Southern Europeans (e.g. North Italians). The source of this East-Asian admixture is closest to present-day Uygurs. However, the true ancient population that was responsible to transmitting East-Asian ancestry into Europe is yet to be determined. It seems likely that the ancestry was brought to Europe via an intermediate population containing East Asian ancestry, such as the Huns or Turkic peoples.

Chapter 6

General Conclusions

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Appendix A

Datasets used

This appendix described the different datasets used in analyses performed in this thesis. It includes datasets of both modern and ancient genomes

A.1 Antonio et al 2019

Samples from Antonio et al (2019) [19]. Looking at the population genomics of ancient Rome.

This dataset consists of 134 shotgun-sequenced individuals from 12,000 years ago to the present day. Coverage ranges from 0.4x - 4x (median 1.05x).

Aligned reads in .bam format were downloaded from the ENA under accession number PRJEB32566.

A.2 Margaryan et al 2020

Samples from Margaryan et al 2020 [20], looking at the population genomics of Vikings.

This dataset consists of 442 shotgun-sequenced individuals from the Bronze Age to Medieval and Early Modern ages. Mean coverage of 1x. X individuals

did not have enough data to estimate recalibration parameters and were excluded from the rest of the analysis.

Aligned reads in .bam format were downloaded from the ENA under accession number PRJEB37976.

A.3 Rivollat et al 2020

Samples from Rivollat et al (2020) [80]. Looking at the population genomics of France and Germany.

This dataset consists of 101 captured individuals from 7000–3000 BCE. years ago to the present day.

Aligned reads in .bam format were downloaded from the ENA under accession number PRJEB36208.

A.4 Brunel et al 2018

Samples from Brunel et al (2018) [93]. Looking at the population genomics of France.

This dataset consists of 52 shotgun sequenced individuals from Mesolithic to the Iron Age.

Aligned reads in .bam format were downloaded from the ENA under accession number PRJEB36208.

A.5 Allentoft et al 2015

Samples from Allentoft et al 2015 [123]. Looking at the population genomics Bronze Age Eurasia.

This dataset consists of 20 shotgun sequenced individuals.

Aligned reads in .bam format were downloaded from the ENA under accession number PRJEB9021.

A.6 Broushaki et al 2016

Samples from Broushaki et al 2016 [124]. Looking at the population genomics Bronze Age Eurasia.

This dataset consists of 1 shotgun sequenced individual.

Aligned reads in .bam format were downloaded from the ENA under accession number xxxx.

A.7 Cassidy et al 2016

Samples from Cassidy et al 2016 [125]. Looking at the population genomics of the Neolithic and Bronze Age migrations to Ireland.

This dataset consists of 4 shotgun sequenced individuals.

Aligned reads in .bam format were downloaded from the ENA under accession number PRJEB11995.

A.8 de Barros Damgaard et al 2018a

The first horse herders and the impact of early Bronze Age steppe expansions into Asia.

Samples from de Barros Damgaard et al 2018a [25].

This dataset consists of 34 shotgun sequenced individuals.

Aligned reads in .bam format were downloaded from the ENA under accession number PRJEB26349.

A.9 de Barros Damgaard et al 2018b

137 ancient human genomes from across the Eurasian steppes

Samples from de Barros Damgaard et al 2018b [126].

This dataset consists of 58 shotgun sequenced individuals.

Aligned reads in .bam format were downloaded from the ENA under accession number PRJEB20658.

A.10 Gamba et al 2014

Genome flux and stasis in a five millennium transect of European prehistory

Samples from Gamba et al 2014 [89].

This dataset consists of 10 shotgun sequenced individuals.

Aligned reads in .bam format were downloaded from the Sequence Read Archive (SRA) under the accession code SRP039766.

A.11 Gunther et al 2015

Genomic diversity and admixture differs for Stone-Age Scandinavian foragers and farmers

Samples from Gunther et al 2015 [127].

This dataset consists of 2 shotgun sequenced individuals.

Aligned reads in .bam format were downloaded from the ENA under acces-

sion number PRJEB9783.

A.12 Hofmanová et al 2016

Early farmers from across Europe directly descended from Neolithic Aegeans

Samples from Hofmanová et al 2016 [82].

This dataset consists of 5 shotgun sequenced individuals.

Aligned reads in .bam format were downloaded from the ENA under accession number PRJEB11848.

A.13 Jones et al 2015

Upper Palaeolithic genomes reveal deep roots of modern Eurasians

Samples from Jones et al 2015 [128].

This dataset consists of 2 shotgun sequenced individuals.

Aligned reads in .bam format were downloaded from the ENA under accession number PRJEB11364.

A.14 Marchi et al 2020

The mixed genetic origin of the first farmers of Europe

Samples from Marchi et al 2020 [129].

This dataset consists of 4 shotgun sequenced individuals.

Aligned reads in .bam format were downloaded from the ENA under accession number PRJEB11364.

A.15 Olalde et al 2014

Derived immune and ancestral pigmentation alleles in a 7,000-year-old Mesolithic European

Samples from Olalde et al 2014 [130].

This dataset consists of 1 shotgun sequenced individual.

Aligned reads in .bam format were downloaded from the ENA under accession number PRJNA230689.

A.16 Sánchez-Quinto et al 2019

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

Samples from Sánchez-Quinto et al 2019 [131].

This dataset consists of 7 shotgun sequenced individuals.

Aligned reads in .bam format were downloaded from the ENA accession number PRJNA230689.

A.17 Seguin-Orlando et al 2014

Genomic structure in Europeans dating back at least 36,200 years

Samples from Seguin-Orlando et al 2014 [132].

This dataset consists of 1 shotgun sequenced individual.

Aligned reads in .bam format were downloaded from the ENA under accession number PRJNA230689.

A.18 Ancient reference dataset

This section describes the generation of the dataset of reference ancient individuals used in Chapters 2, 4 and 5.

The following steps were used to generate the data:

1. Each `.bam` was processed with `PicardTools ValidateBam` [28] task to ensure no files were corrupted or contained incorrect read group information.
2. Each `.bam` file was processed with `atlas` (version 1.0, commit f612f28) pipeline [23] (<https://bitbucket.org/wegmannlab/atlas/wiki/Home>). For `.bam` file, I estimated post-mortem damage (PMD) patterns using `atlas estimatePMD` task. Recalibration parameters were then estimated using `atlas recal` task. Finally, both the recalibration and PMD parameters were given to the `callNEW` task which produces genotype calls and genotype likelihood estimates for each downsampled and full coverage `.bam`. For this stage, I made calls at the 77,818,345 genome-wide positions present in the phase 3 thousand genomes project [29]. This was done to reduce the risk of calling false-positive non-polymorphic sites. This resulted in a `.bcf` file for each ancient sample.
3. All `.bcf` files were split into chromosomes and all samples from the same chromosome were merged. Imputation and phasing was performed with `GLIMPSE` (version 1.1.1). I followed the steps laid out in the `GLIMPSE` tutorial (https://odelaneau.github.io/GLIMPSE/tutorial_b38.html). First, I used `GLIMPSE_chunk` to split up each reference chromosome into chunks, keeping both `-window-size` and `-buffer-size` to 2,000,000, their default settings. Across all chromosomes, this produced 936 chunks of an average 2.99Mb long. I used the b37 genetic map supplied by `GLIMPSE` for the `-map` argument.

Each chunk was then imputed separately using `GLIMPSE_phase` using the same 1000 genomes dataset as a reference. Default settings and the supplied b37 genetic map were used. This stage both imputes missing genotypes and generates a set of haplotype pairs which can be sampled from in a later step to produce phased haplotypes.

`GLIMPSE_ligate` was then used to merge the imputed chunks back to form single chromosomes using the default settings and the supplied b37 genetic map.

Haplotypes were then sampled using `GLIMPSE_sample` to produce a .vcf with phased haplotypes for each individual, again using default settings and the supplied b37 genetic map.

Consequently, the output of GLIMPSE is i) unphased genotype calls with posterior genotype likelihoods and ii) phased haplotypes.

4. Finally, the posterior genotype likelihoods and phased haplotypes were combined to generate ChromoPainterUncertainty output using a custom script (https://github.com/sahwa/vcf_to_chromopainter).

A.19 30x 1000 genomes dataset

Samples from [35].

This dataset consists of 3,202 modern individuals from 26 worldwide populations, sequenced to a targeted depth of 30x coverage. The downloaded dataset was aligned to the gr38 reference genome.

Samples were downloaded to the UCL Computer Science cluster by myself from the ftp mirror.

The following steps were taken to process the data before being used as an imputation reference.

1. Filtered such that SNPs with only 2 alleles were retained
2. Performed a liftover to hg19 using LiftOverVcf from picard tools [28]
3. Filter again for SNPs with only 2 alleles
4. Phase using shapeit4, using the ‘sequencing’ parameter and setting –pbwt-depth 4.
5. Remove duplicated SNPs using bcftools norm [74]
6. Use Beagle’s conform-gt utility to ensure reference alleles were consistent with the previous 1000 genomes build. This was done because all previous datasets I have compiled were also conformed to the previous 1000 genomes build.

A.20 Human Origins dataset

This dataset consists of 560,420 SNPs and 5998 individuals from 509 worldwide populations. It has a particularly large number of samples from West and East Africa; in particular, Cameroon, Ethiopia, Nigeria and Ghana.

A.20.1 Processing

Only bi-allelic SNPs were retained. To ensure that all datasets, ancient and modern, can be merged together without the confounding effects of strand flips, I then used conform-gt (<https://faculty.washington.edu/browning/conform-gt.html>) to align all alleles to the same strand as the 1000 genomes reference, keeping all parameters as default. Any genotypes which had a genotype likelihood of below 0.990 were set as missing.

Data was phased use `shapeit4` [31], setting `-pbwt 8` and keeping all other parameters as default. The 1000 Genomes was used as as reference (section [?]). Sporadic low quality missing genotypes were imputed.

A.21 MS POBI HellBus dataset

Multiple Sclerosis (MS), People of the British Isles (POBI), Hellenthal and Busby (HB) / MS POBI HellBus contains a total of 14,795 individuals from 211 worldwide populations.

Samples from Sawcer et al (2011) [133] (10299 individuals from 15 pops), Leslie et al 2015 [42] (2039 individuals from 35 pops) and Busby et al (2457 individuals from 161 pops).

Individuals from MS populations USA, Canada and New Zealand were all removed as the individuals were not native to that country.

The following steps were taken to process the data

1. Filtered such that SNPs with only 2 alleles were retained
2. Phase using shapeit4 [31] setting `-pbwt-depth 8`.
3. Remove duplicated SNPs using bcftools norm [74]
4. Use Beagle's conform-gt utility to ensure reference alleles were consistent with the previous 1000 genomes build. This was done because all previous datasets I have compiled were also conformed to the previous 1000 genomes build.

Appendix B

Another Appendix About Things

Some terms that are helpful to define

- linked
- unlinked
- all-v-all

Appendix C

Colophon

This document was produced using the UCL thesis L^AT_EX template (<https://github.com/UCL/ucl-latex-thesis-templates>).

This document was set in the lmodern typeface using L^AT_EX and BibT_EX, composed with a text TexMaker on Linux. `microtype` was also used.

All figures were generated using `ggplot2` using `theme_light()`.

The final version of the thesis can be found at <https://github.com/sahwa/thesis>.

Appendix D

Supplementary figures

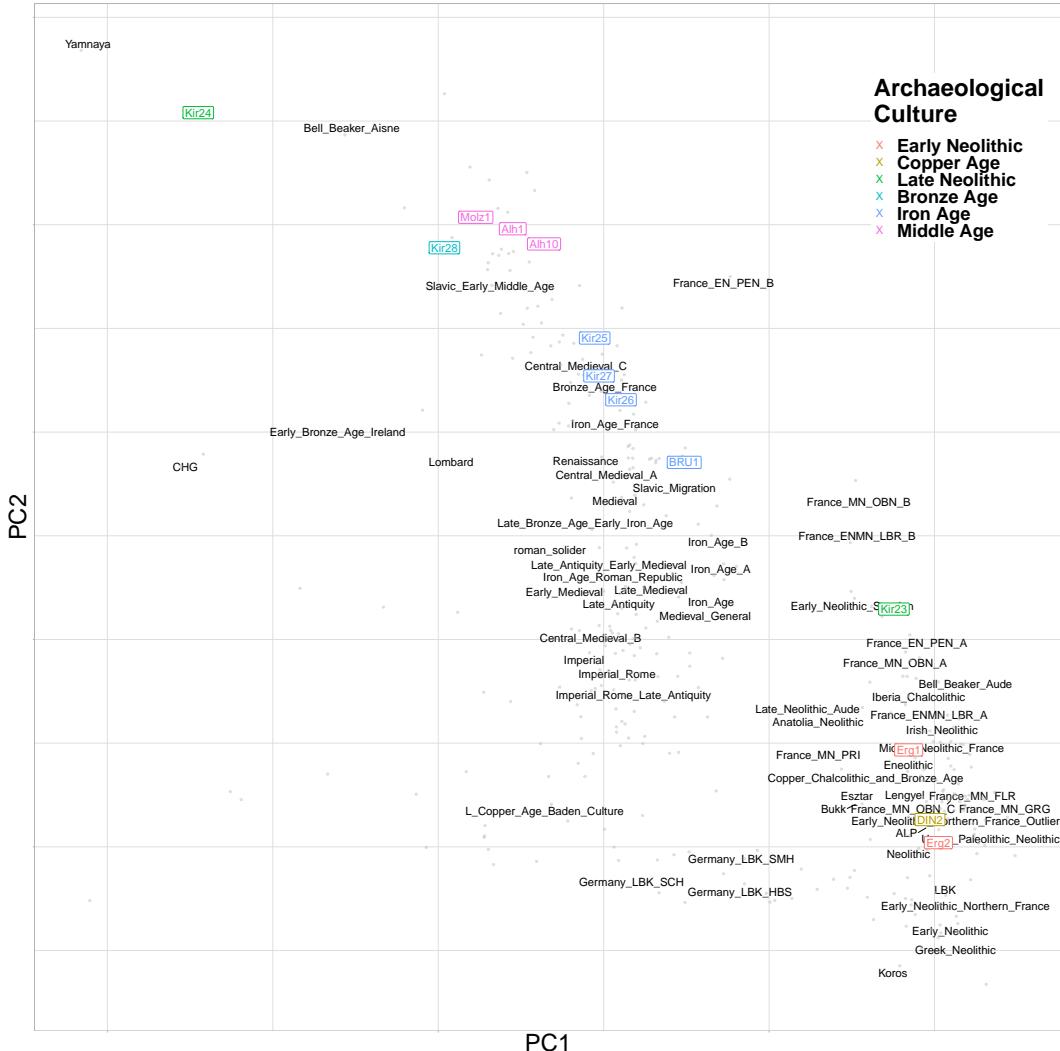


Figure D.1: Principle component analysis of genotype matrix using plink2. Grey points indicate principle component coordinates for each sample. Black text indicated mean principle component coordinates for all individuals within that group. Coloured labels represent newly sequenced ancient samples.

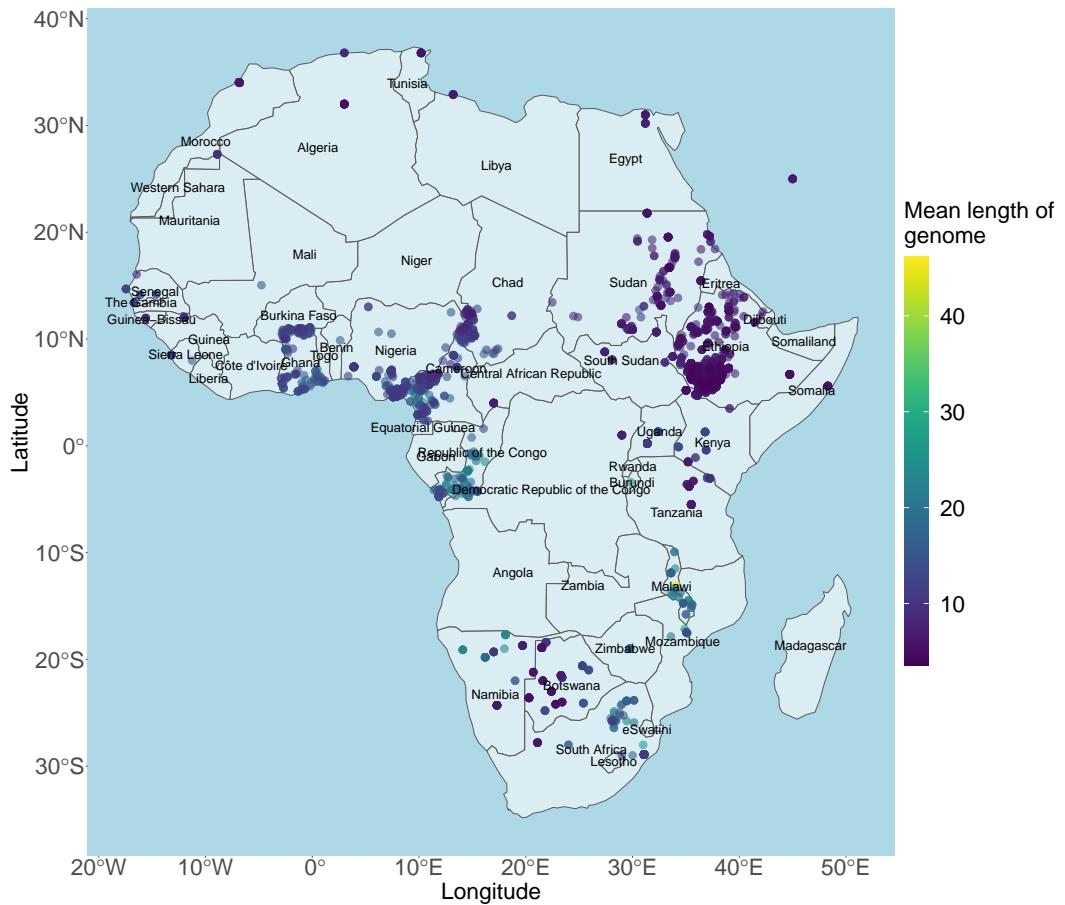


Figure D.2: Principle component analysis of genotype matrix using plink2. Grey points indicate principle component coordinates for each sample. Black text indicated mean principle component coordinates for all individuals within that group. Coloured labels represent newly sequenced ancient samples.

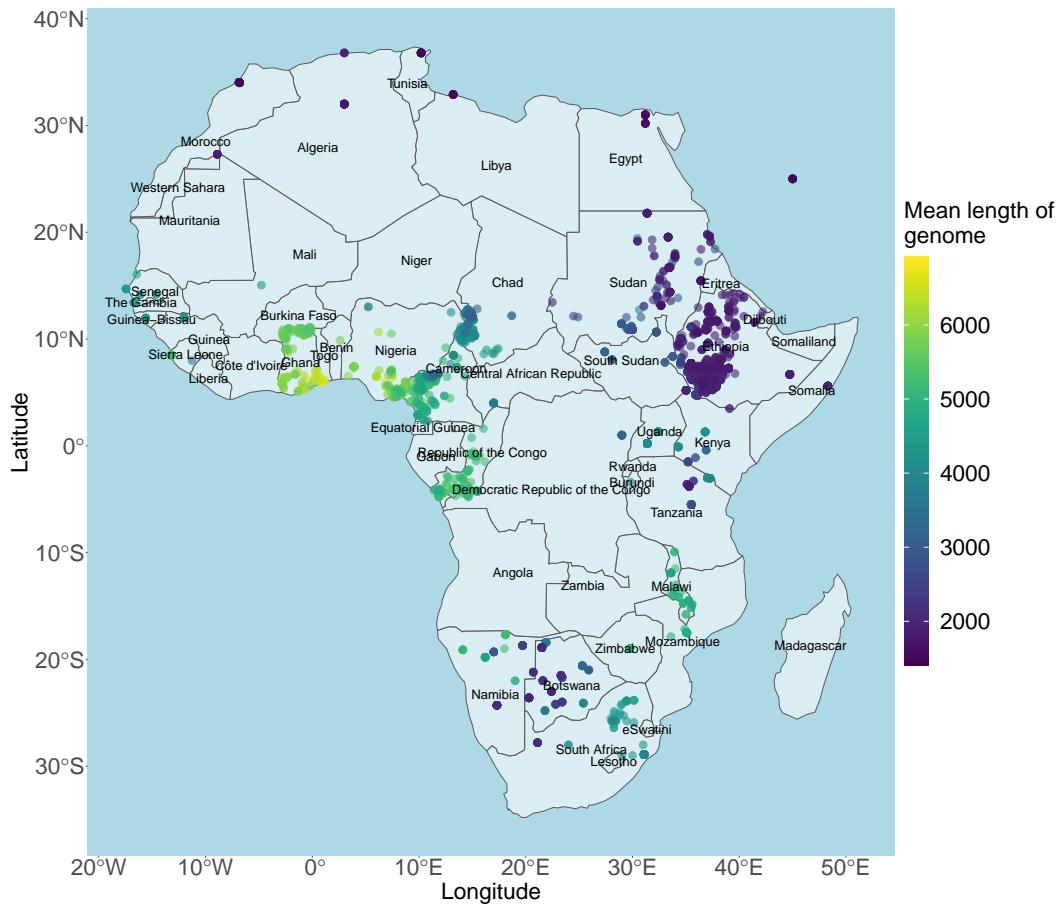


Figure D.3: Principle component analysis of genotype matrix using plink2. Grey points indicate principle component coordinates for each sample. Black text indicated mean principle component coordinates for all individuals within that group. Coloured labels represent newly sequenced ancient samples.

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