Discerning Ancestry-Related Assortative Mating from Migration by their Genomic Imprints upon Admixed Populations

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Many human populations throughout history have become socially stratified based on perceived race. Assortative mating, the tendency to mate with those similar to you, is a key reason for this. When ancestral origin of different regions of a genome is mapped out, the effects of this non-random admixture can be inferred by the distribution of the size of fragments consecutively assigned to the same ancestry. However, differentiating assortative mating from migration is difficult since past migration manifests similarly in the genomes of a population. Here I show how significant modern-day assortative mating can be detected in and compared between admixed North and South American populations. This empirical evidence of assortative mating may bring the validity of certain genome-wide association studies and research of the genomic impacts of migration into question. Furthermore, I help to lay the groundwork for a technique that can integrate migration data with genomic data to accurately quantify and timestamp past assortative mating. If successful in doing so, we can hopefully unveil the historical context for modern-day ancestry-related social straitification present in populations throughout the world, and perhaps track it in real time.

Contents

1 Introduction

Positive assortative mating, a phenomenon wherein individuals are more likely to mate with those phenotypically similar to themselves, is widely accepted to occur in human populations (Norris et al., 2019). This has the potential to alter population structure by introducing social stratification and, in turn, create social constructs upon which further assortative mating can be based, such as wealth, class or social policies (Risch et al., 2009).

From a genetics standpoint, this multigenerational non-random admixture between genetically distinct groups leaves a genomic imprint in the individuals comprising the population, in stark contrast to populations more closely following Hardy-Weinberg equilibrium (HWE) (Zaitlen et al., 2017). However, the genomic imprint on the population structure left by either sociocultural barriers or geographical barriers limiting admixture is difficult to discern. Afterall, large scale migration of a population will genetically manifest itself similarly to the change of societal rules or norms that condition social interaction, such as the revocation of racial segregation policies.

Single nucleotide polymorphisms (SNPs) can be used as indicators of ancestry (Risch et al., 2009). Population genomics techniques allow us to generate a large array of SNPs which can be analysed using local ancestry inference to map ancestries to positions and regions along the genome, after which further analysis can indicate past assortative mating in a population (Schubert et al., 2020).

One such analysis is that of continuous ancestry tract lengths: the lengths of genomic regions consecutively assigned to the same ancestry. Looking at the distribution of these lengths, the ancestry to which they belong and the overall ancestry proprtion of individuals within a population can indicate how long ago the admixture occurred and to what extent. Recombination of the DNA of admixing individuals leads to a decrease in continuous ancestry tract lengths, as those within the parents' genomes interupt one another upon recombination. Hence, admixture more generations ago will manifest as distributions of shorter continuous ancestry tracts and vice versa (Gravel, 2012).

Genotype frequency is another indicator of population admixture; one would expect a more admixed population to have higher heterozygous genotype frequencies at a given position. While this alone does not inherently indicate assortative mating, the extent to which the observed genotype frequency deviates from what would be expected under HWE can also be considered. The assortative mating index (AMI) quantifies the relative local ancestry homozygosity-to-heterozygosity ratio at a given position based on this concept, which can be used as a proxy for the extent of assortative mating at said position (Norris et al., 2019).

HWE is commonly used in population genomics as a quality check for genetic markers in genome-wide association study (GWAS) - SNPs chosen for being particularly informative for certain pathological research - whereby alleles with frequencies deviating too far from it are removed and deemed sequencing misreads (Linares-Pineda et al., 2012). This does not take into account stratificatiom, present in most if not all societies, within the populations studied therein. Showing that allelic deviation from HWE is not an artefact but rather an intrinsic quality may serve as a warning against this practice.

Populations of the Americas such as Colombia, Barbados, Mexico or the US provide apropriate and well-researched case studies integrating migration, admixture and assortative mating.

Many of such populations have different but connected histories: a Native American population is colonised by Europeans; the Native population shrinks due to war, hard labour and disease, while the European population grows via migration. These phenomena continue such that African slaves are transported to the region as a source of additional labour; after which the population continues evolving with the lingering impacts of colonialism (Bryc et al., 2010; e Silva et al., 2020; Mas-Sandoval et al., 2019).

These North and South American populations are far from the only examples of where migration and assortative mating coincide and can be studied, indeed most human populations are the result of admixture between multiple populations. However, the three source populations giving rise to the admixed population - African, European and Native - being genetically distinct facilitates the identification of local ancestry fragments and enables the study of the complex admixture process.

Analysing the length of the local ancestry fragments, it is possible to evaluate both the admixture dates and the strength of the ancestry-related assortative mating. Said assortative mating can be understood as the degree of impermeability of the socioeconomic and cultural barriers between subgroups of the admixed population with differentiable genetic ancestries. Further understanding and ideally quantifying ancestry-related assortative mating, and using it as a proxy for ancestry-related social stratification, will not only help us better understand how such stratification historically and presently influence mating behaviours in the Americas, but could also be used to track or predict it in present and future admixed populations.

To accurately estimate the extent of assortative mating in a population using genomic techniques, the genomic impact of migration on said population must be accounted for, despite them being difficult to differentiate. Previous research has either studied genomic impact of migration while assuming otherwise random admixture (Borda et al., 2020; Gravel, 2012; Norris et al., 2020), or studied assortative mating while assuming a single pulse of migration from each immigrating ancestry (Norris et al., 2019; Risch et al., 2009; Zaitlen et al., 2017). However, for reasons outlined, studies on the effects of migration on population genomics must consider assortative mating, and when studying assortative mating one must consider migration as a continuous process rathen than a single event. Equally, comparing measured assortative mating levels of different populations and cross-referencing this with their histories and current socioeconomic climates could yield interesting insights as to casues and long-term effects of ancestry-related social stratification.

Hence, the aims of this project are twofold. Firstly, to use genomic data from admixed populations of the Americas to explore different analytical methods designed to unveil non-random admixture in a population. This will enable me to compare these methods by their potential to distigunish between migration and assortative mating as sources for this non-random admixture. Secondly, to use the results of these analyses to compare the admixed populations by the level of assortative mating revealed. My hypotheses are that each population will exhibit significant positive assortative mating, and that the level of said assortative mating in each population will be significantly different to that of the others.

Only by reconciling migration and assortative mating can we confidently infer assortative mating from genomic data, and use this to draw conclusions about past and detect trends of future ancestry-related social stratification.

2 Methods

2.1 Studied Populations

For the initial analyses, all African, European and American populations from the 1000 Genomes Project (1KGP) and the Human Genome Diversity Project (HGDP) were used **Table 1**, with the exception of the Russian and Finnish populations. These were excluded owing to minimal colonial-era migration to the Americas from these populations, alongside the genetic similarities between these populations, Siberans and, by extension, Native Americans.

Table 1: Details of the populations used throughout this study. Populations abbreviated as three capitalised letters are from the 1000 Human Genome Project dataset, while full-word abbreviated populations are from the Human Genome Diversity Project dataset. The number of samples used from each population is denoted by n.
*The Tuscan and Yoruba populations comprise samples from both datasets.

Superpopulation	Population	Abbreviation	n
Admixed	African Ancestry in Southwest USA	ASW	61
	African Caribbean in Barbados	ACB	96
	Colombian in Medellin, Colombia	CLM	94
	Mexican Ancestry in Los Angeles, California	MXL	64
	Peruvian in Lima, Peru	PEL	85
	Puerto Rican in Puerto Rico	PUR	104
African	Bantu in Kenya	BantuKenya	11
	Bantu in South Africa	BantuSouthAfrica	8
	Biaka in Central African Republic	Biaka	22
	Esan in Nigeria	ESN	99
	Gambian in Western Division, The Gambia	GWD	113
	Luhya in Webuye, Kenya	LWK	99
	Mandenka in Senegal	Mandenka	22
	Mbuti in Democratic Republic of Congo	Mbuti	13
	Mende in Sierra Leone	MSL	85
	San in Namibia	San	6
	Yoruba in Nigeria	YRI/Yoruba*	129
European	Basque in France	Basque	23
	Bergamo Italian in Bergamo, Italy	BergamoItalian	12
	British in England and Scotland	GBR	91
	Northern and Western European Ancestry in Utah	CEU	99
	French in France	French	28
	Orcadian in Orkney	Orcadian	15
	Sardinian in Italy	Sardinian	28
	Iberian in Spain	IBS	107
	Toscani in Italy	TSI/Tuscan*	115
Native American	Colombian in Colombia	Colombian	7
	Karitiana in Brazil	Karitiana	12
	Maya in Mexico	Maya	21
	Pima in Mexico	Pima	13
	Surui in Brazil	Surui	8

2.2 Data Preparation with BCFtools

Using BCFtools v1.9, the 30x coverage 1KGP and high-coverage HGDP datasets were merged, and all populations except those listed in **(Table 1)** were removed. All C→G, G→C, A→T and T→A SNPs were filtered out as they are harder to assign and are hence prone to error (Danecek et al., 2021). SNPs were further filtered with a minor allele frequency threshold of 5%, as to reduce the dataset and remove rare and thus uninformative SNPs. Following this, all 22 filtered VCF files,

one per autosome, were indexed for phasing.

2.3 Haplotype Estimation with SHAPEIT4

Phasing was carried out using SHAPEIT v4.2.0, which efficiently assigns haplotype estimates for each genotype by cross-referencing the genomic region in question with the corresponding region of a pre-phased reference panel and of the other genomes being phased (Delaneau et al., 2019). The programme was run using the B38 genetic map recommended by the developers and default parameters, plus an apropriate high-coverage phased reference genome from the 1KGP website (see: **Data and Code Availability**) to inmprove haplotype estimation accuracy. The individually phased chromosomes were then merged into a single VCF file with BCFtools.

2.4 Ancestry Estimation with PLINK & ADMIXTURE

Linkage disequilibrium pruning was performed with PLINK v2.0 on the genomes in VCF format, which creates a subset of largely independent SNPs - thereby significantly reducing the computational power needed for subsequent analyses with minimal information loss - before converting the pruned dataset to PLINK format (Purcell et al., 2007). These SNPs form the basis of this study.

The programme ADMIXTURE v1.3.0 used cluster analysis and principal component analysis to estimate the proportions of African, European and Native American ancestry for each remaining sample, with default parameters and three ancestries to be detected (Alexander et al., 2009).

2.5 Local Ancestry Inference with RFMIX v2

The ADMIXTURE outputs were subsequently used to filter out all significantly admixed samples, with a minimum threshold of 99% African, European or Native American ancestry. This subsetting was executed using BCFTools, yielding a subset VCF of >99% non-admixed samples was used as a reference panel for local ancestry assignment with the programme RFMIX. A query subset was created correspondingly, containing all samples in the "Admixed" superpopulation in **(Table 1)**.

RFMIX v2.03-r0, based on concepts developed in RFMIX v1, assigns ancestries to segments of an individual's genome, which not only yields ancestry proportions as with ADMIXTURE, but also effectively maps out each genome in terms of each genomic region's estimated ancestry or origin. It does this by progressively modelling ancestry along each chromosome using discriminant random forests, conditional random field modelling and observed haplotype sequences of ancestry infered from an input reference panel (Maples et al., 2013).

The RFMIX run was performed using the aforementioned query and reference VCF files, and a sample map linking the sample codes to their respective populations. Parameters used were three run-throughs of the algorithm and 20 generations, before which, assuming an average generation length of 25 years, no known European-Native American admixtire had taken place.

2.6 Assortative Mating Index Calculation

One measure of assortative mating is the assortative mating index (AMI), which takes a log odds ratio of the relative local ancestry homozygosity and heterozygosity:

$$AMI = ln\left(\frac{hom^{obs}/hom^{exp}}{het^{obs}/het^{exp}}\right) \tag{1}$$

Three ancestries are being investigated, hence expected homozygous and heterozygous allelic frequencies can be thought of in terms of the biallelic (**Equation 2**) or triallelic (**Equation 3**) Hardy-Weinberg models (Norris et al., 2019):

$$(x + \bar{x})^2 = x^2 + 2\bar{x}x + \bar{x}^2 \tag{2}$$

$$(a + e + n)^2 = a^2 + e^2 + n^2 + 2ae + 2an + 2en \tag{3}$$

The left side of each of these models are haplotype frequencies while the right sides are genotype frequencies, each side of the equation summing to one. In the triallelic model, a, e and n are the initials of the ancestry they represent, while x and $\bar{x}$ in the biallelic model correspond to a given ancestry - African, European or Native - and all other ancestries respectively. Hence, while AMI is calculated only once using the triallelic model, the AMI using the biallelic model must be calculated three times: once with respect to each ancestry. For example, with respect to African ancestry, the homozygous genotype would be both African alleles or both non-African alleles, and the heterozygous genotype would be one African allele and one allele of one of the other ancestries.

The outputs of RFMIX v2 were analysed by a series of R Studio scripts I created for this project (see: **Data and Code Availability**). Firstly, the forward-backward (.fb.tsv) ouput files were read by the script "rfmix.fb.tsv_genotype_assign_HPC.R". These files contain the estimated haplotype probabilities at each genolmic position for each sample. The script then assigns the genotype for each genomic position in each sample, with a probability threshold of 0.9, and returns the frequencies of each of the six triallelic genotypes at each position across samples as a table. This genotype frequency table is then read by the script "rfmix.fb.tsv_genotype_analysis.R", before calculating the triallelic AMI, and the three biallelic AMIs with respect to each ancestry, at each position.

2.7 Continuous Ancestry Tract Length Analysis

Ancestry assignments of lower certainty in the forward-backward file, using the 0.9 probability threshold, have the potential to fracture continuous ancestry tracts thereby completely alter the distribution of their lengths. Hence the .msp.tsv RFMIX output files were used instead, equivalent to the forward-backward files but with automatic haplotype assignment to haploytype with highest estimated liklihood.

To generate the fragment length distributions, the script "rfmix.msp.tsv_window_size.R" reads the .msp.tsv files, sums the length of consecutive genomic windows assigned to the same ancestry, and appends the lengths to the vector containing the lengths of other fragments corresponding to the fragment's ancestry and population. The script "rfmix.msp.tsv_inbred_window_size.R" works similarly, but generates fragment length distributions of consecutive homozygous genotype assign-

ments, rather than haplotype assignments.

2.8 Timeline of Admixture Estimation with TRACTS

TRACTS is a software for modelling migration histories using ancestry tracts data, incorporating the theory of time-dependent gene-flow and correcting for chromosomal end effects and haplotype assignment errors. In doing so, it predicts how many generations prior to the query genomes the migration events bringing the different populations together occured (Gravel, 2012).

The software uses the .bed file format as input, a file output of the original RFMIX but not of RFMIX v2, hence I created a script to convert .msp.tsv to .bed, "msp2bed_conversion.R". This merges together each chromosome from the 22 .msp.tsv files, and merges each consecutive intrachromosomal fragment - pre-defined by RFMIX - of the same ancestry into single fragments whereby adjhacent fragments can be of vastly different lengths and always different assigned ancestries. It then recalculates each cell based on this mergeing of fragments assigned to the same ancestry, reshuffles and reformats the columns, and saves one .bed file per query sample, each .bed file containing fragments constituing the entire genome of one individual, as required to run TRACTS.

Because in each of the admixed query populations there was initial admixture between Native Americans and Europeans populations, followed by African and further European ancestry being added to the gene pool, none of the models provided by TRACTS were entirely apropriate. I therefore adjusted the provided four population model, which assumes admixture of two initial populations and subsequently two further populations with three migration events, to instead assume initial admixture between two populations and subsequent admixture with one of those two populations (European) and a third population (African). Said adjusted model is encoded in the Python 2 script "models_4pop.py", which is run by "taino_ppxx_xxpp.py" for each admixed query populations with 25 bootstraps.

The _mig file outputs of TRACTS contain what proportion each newly introduced ancestry contributes to the query population after each migration event, and how many generations ago that migration event occurred. The script "tracts_mig_plots.R" uses this data to calculate the estimated relative proportion of the three ancestries during each of the past 25 generations for each query population.

3 Results

3.1 Ancestry Proportion

Pruning led to the dataset being reduced to 4,111,226 SNPs per sample. ADMIXTURE was used on these SNPs to estimate ancestry proportion of three ancestries - African, European and Native - for all 1690 individuals represented in **Table 1**.

The averaged output for each of the 31 populations is visualised in **Fig. 1**, which displays Peruvians and LA Mexicans as predominantly of Native ancestry and minimially African; Colombians and Puerto Ricans as predominantly European but more Native than African; and Barbadians and African Americans from US Southwest (ASWs) as predominantly African with minimal Native ancestry.

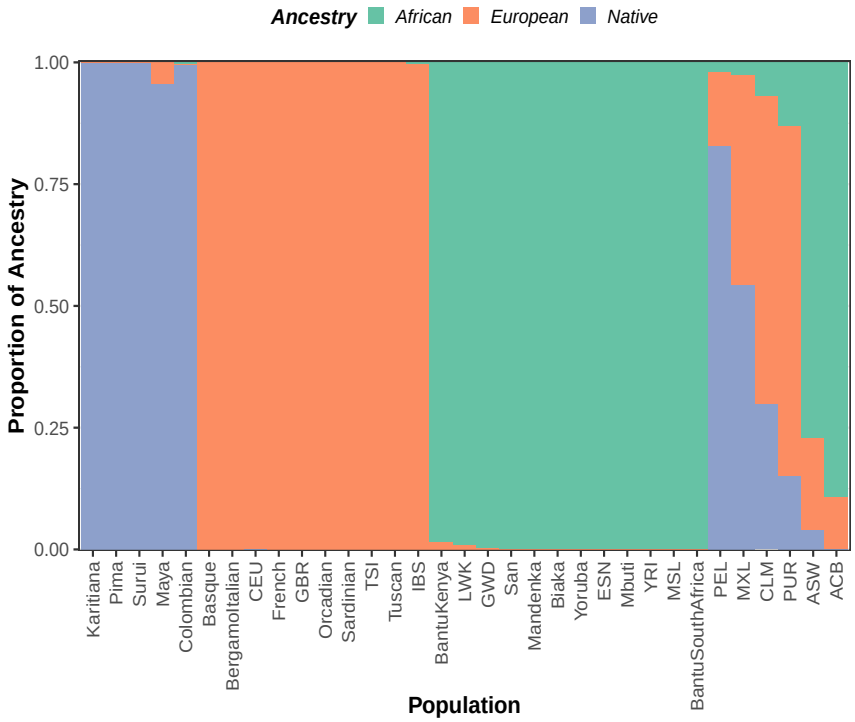

Figure 1: Stacked barplots showing the proportions of the three ancestries of each reference or query population used throughout the study, generated by ADMIXTURE. Genomic data from individuals of selected populations from the 1000 Genomes Project and the Human Genome Diversity Project were processed and subjected to ADMIXTURE, the output of which was averaged for all individuals of a given population. Populations 1-5 are Native, 6-15 are European, 16-27 are African, and 28-33 are admixed from the Americas.

The distribution of ancestry proportions on the individual level within these six admixed populations is shown in **Fig. 2**, which largely corresponds with **Fig. 1** while suggesting aproximately 25% of LA Mexicans and Colombians have no African ancestry, fewer than 5% and 50% of Barbadians and ASWs respectively have Native ancestry, and that only around 20% of Peruvians have African ancestry while around 25% of them are of exclusively Native ancestry.

These individual-level ancestry proportion distributions are further visualised in **Fig. S1**, with the distribution for each population of a given ancestry displayed side-by-side in box plots. All distributions were different at the 5% significance level, except for Barbadian and Peruvian European ancestry proportion distributions. However, their Native and African ancestry distributions contrast starkly, lending credence to the assumption all six admixed populations have entirely different ethnological structures.

Following the admixture run, the 25 reference populations were filtered to remove all samples with less than 99% of the corresponding ancestry. This left a reference panel of 72, 507 and 550 people of 99% or more Native, European and African ancestry respectively for use in the local ancestry inference by RFMIX of the 504 query samples from the admixed populations.

3.2 Assortative Mating Index

One of the outputs of RFMIX is equivalent to that of ADMIXTURE, and a comparison of their relative performance on the 1690 studied individuals is shown in **Fig. S2**. Briefly, RFMIX tends to give lower African ancestry proportion estimates than ADMIXTURE in those both deem to have higher African Ancestry, and higher European ancestry proportion estimates in those both deem

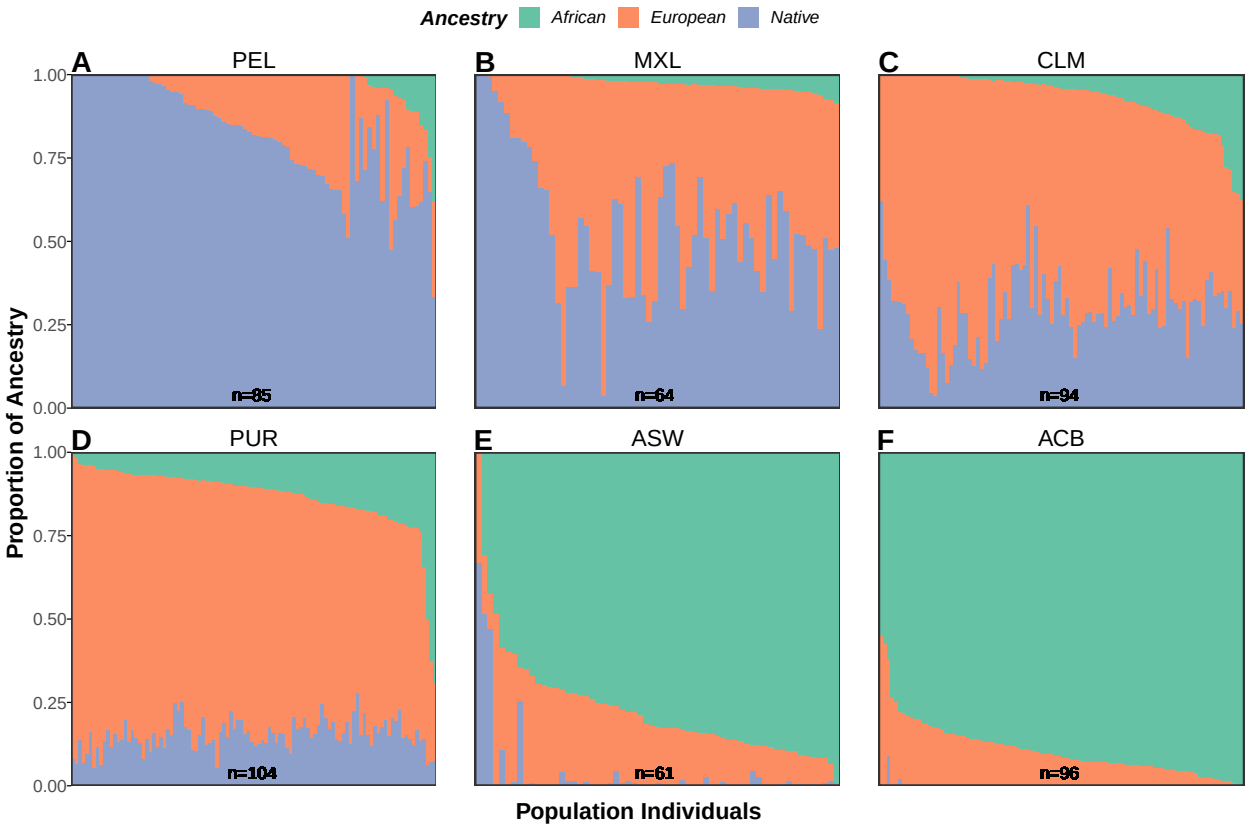

Figure 2: Stacked barplots showing the proportions of the three ancestries of each individual comprising the six query admixed populations, generated by ADMIXTURE. Genomic data from individuals of the six from the 1000 Genomes Project and the Human Genome Diversity Project were processed and subjected to ADMIXTURE. The number of individuals comprising each poppulation is denoted by n, and inviduals are ordered within each respective admixed population's plot by increasing African and then European ancestry.

to have lower European Ancestries.

The main RFMIX output was used to calculate assortative mating index values for each SNP in each population. The triallelic AMI values for each position and population are plotted in **Fig. 3**. In a population without assortative mating, we would expect the mean AMI value to be zero. With a sample size of 4,111,226 SNPs, and the standard deviations being of similar sizes to the corresponding means, the standard errors of the means are negligable and hence the sample means are accurate estimates of the true means. Based on this, we can see all means are significantly higher than zero, indicating positive assortative mating in all admixed populations.

Wilcoxon tests were performed to also ascertain whether the AMI distribution of each population are significantly different from the other populations, which was confirmed to be the case (**Fig. S4A**).

The same analyses were carried out for the biallelic ancestry-specific AMI values. With the same large sample size, the distribution of each population is significantly higher than zero for all three ancestries, confirming that assortative mating has occurred in each population with respect to all three ancestries.

Wilcoxon tests were then performed to compare the AMI distributions of each ancestry by population and of each population by ancestry (**Fig. S4B** and **C** respectively). With the exception of European-specific AMI distriutions for Puerto Rico and Peru, all combinations of ancestries or populations were significantly different.

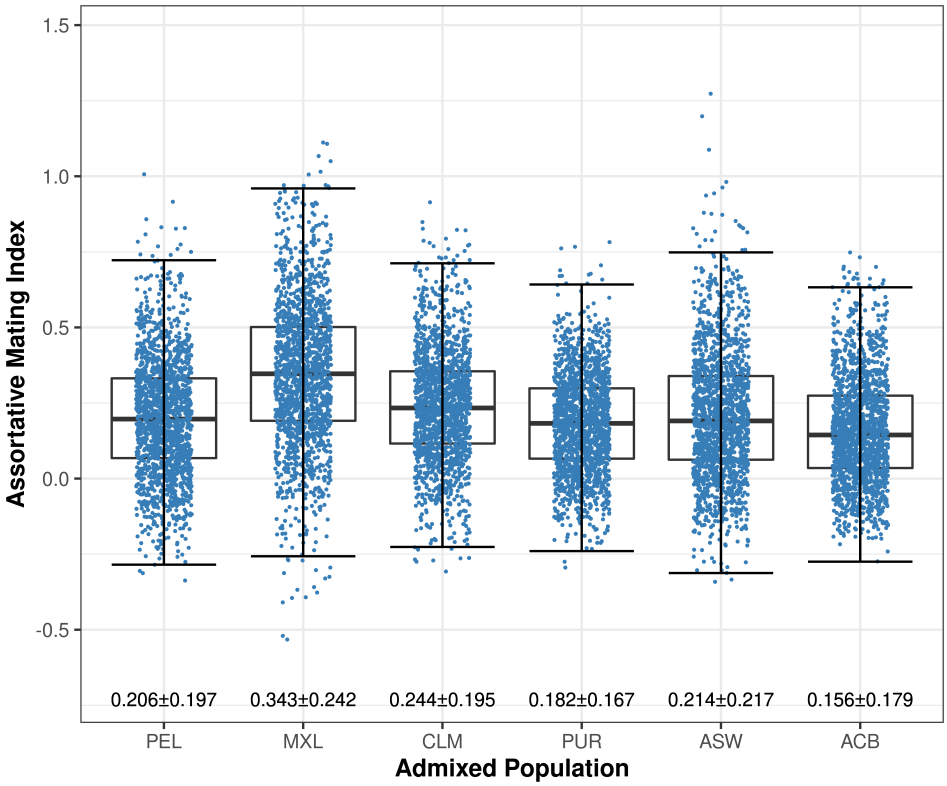

Figure 3: Comparative box plots displaying the distribution of the triallelic assortative mating index calculated for each studied single nucleotide polymorphism for each admixed population. The boxes highlight the upper quartile, median and lower quartile values of the distribtuion, while the whiskers signify the last data point within the closest quartile value plus 150% of the interquartile range. Mean ± standard deviation is given beneath; the standard error of the mean is negligable owing to the sample size of 4,111,226. Horizontal jitter is used to better display the distribution.

To test whether mean ancestry-specific AMI value is correlated with or driven by mean ADMIXTURE-estimated ancestry proportion they were plotted for each admixed population (**Fig. S3**), but no significant correlation was found (p-value = 0.133).

3.3 Continuous Ancestry Tract Lengths

The final use of the RFMIX output was analysisng the lengths of continuous anestry tracts. Displaying the haplotype continuous ancestry tracts in a histogram allows visual comparison between the tract length distriutions of the different ancestries (**Fig. 5A**), while box plots better visualise descriptive statistics of the data (**Fig. S5**).

As would be expected, there's a clear correlation between the relative heights and x-axis positions of the distributions in a given population and the corrrespinding mean ancestry proportion. Skewed distributions, such as the right-skewed African distributions of the ASW and ACB plots, suggest some form of deviation from HWE but whether they are caused by migration, assortative mating or some other phenomenon is unclear.

A supplementary approach is finding and plotting homozygous continuous ancestry tract lengths, as in **Fig. 5B**. This exagerates HWE deviations, and provides additional peaks to some of the distributions. These peaks are more informative than just skewness: they show different tract length distributions of the same ancestry that have been merged, essentially representing two populations of the same ancestry to merging into one. Hence significant same-ancestry migration is the likely cause of corresponding skewness in the haplotype continuous ancestry tract length

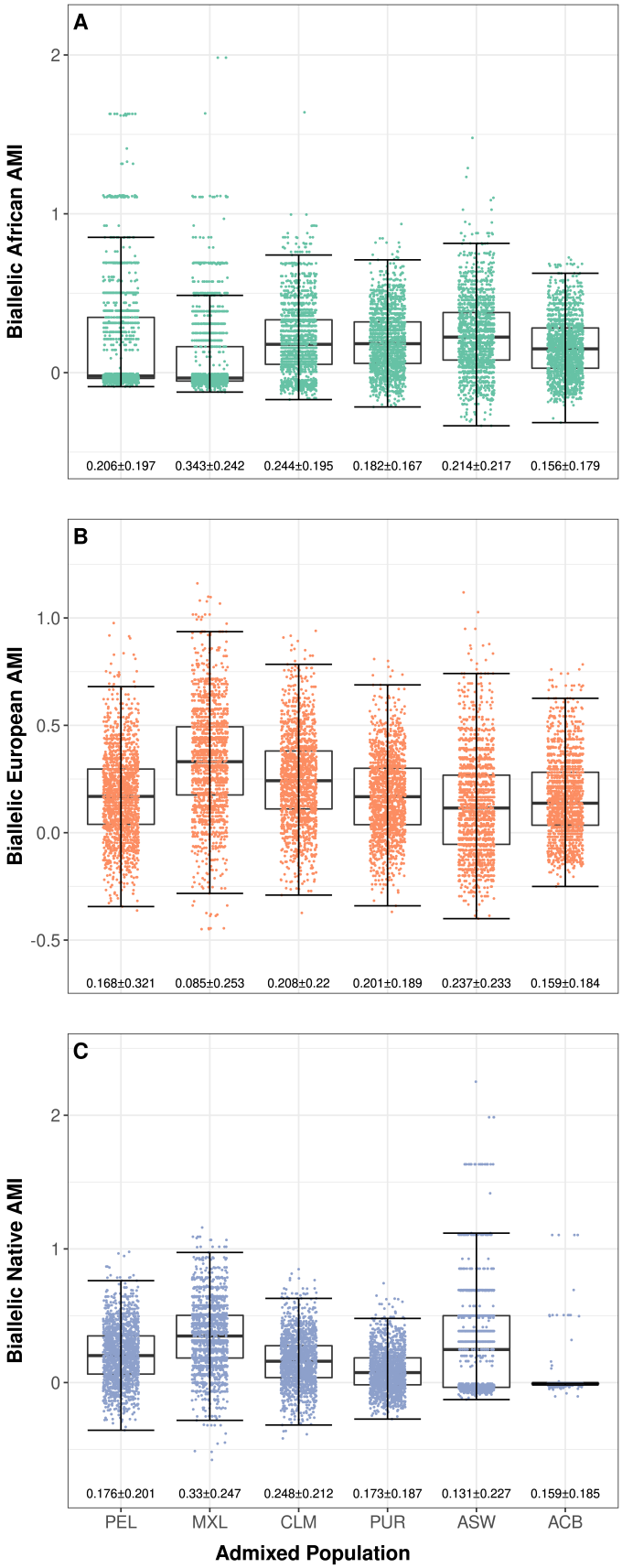

Figure 4: Comparative box plots displaying the distribution of the biallelic ancestry-specific assortative mating indicies calculated for each studied single nucleotide polymorphism for each admixed population. The boxes highlight the upper quartile, median and lower quartile values of the distribtuion, while the whiskers signify the last data point within the closest quartile value plus 150% of the interquartile range. Mean ± standard deviation is given beneath; the standard error of the mean is negligable owing to the sample size of 4,111,226. Horizontal jitter is used to better display the distribution.

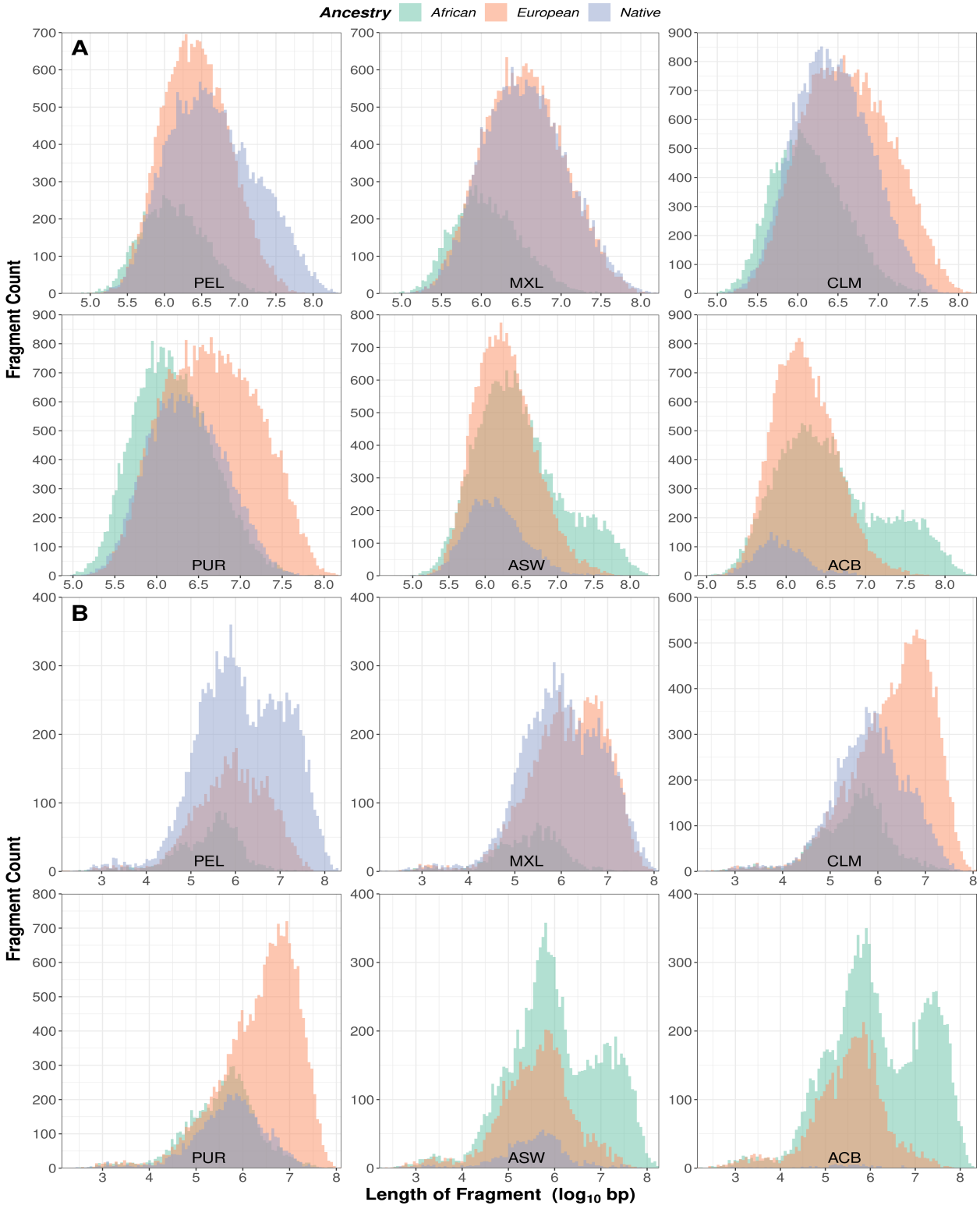

Figure 5: Histograms of continuous ancestry tract lengths of each of the three ancestries for each admixed population. Fragment lengths are measured in base pairs in log10 scale, and are seperated into 100 bins in each plot. Fragment length is considered either the number of consecutive haplotype asignments of a given ancestry on a single strand (A), or the number of consecutive homozygous genotype asignments of a given ancestry on both strands (B).

visualisations, for example with ASW and ACB.

Less intense right skewness, such as with European ancestry in the ASW population, could indicate either minor and sustained European migration, or European assortative mating, where at least some of the European population disproportionately interbred thereby preserving longer

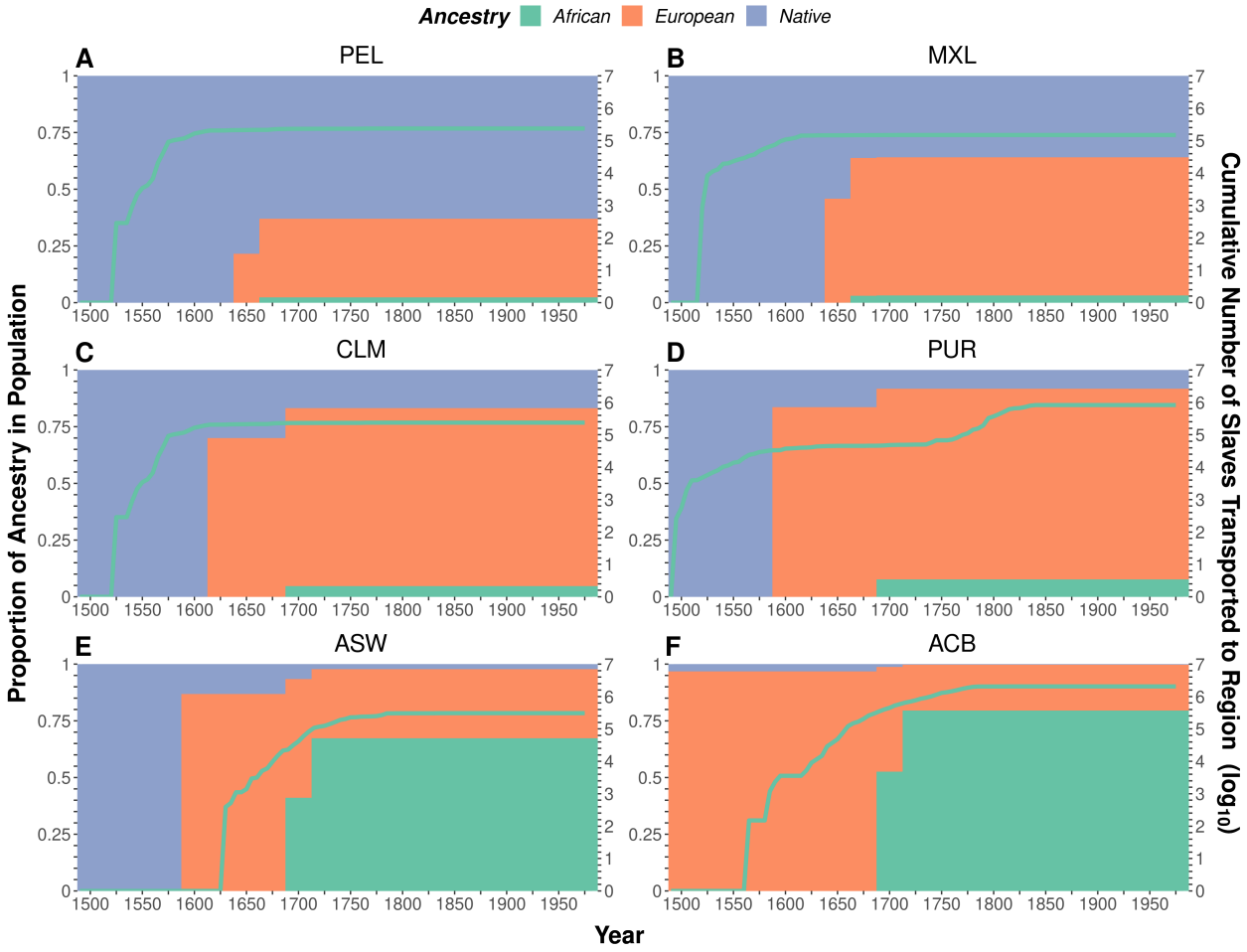

Figure 6: Number of slaves transported to the general regions of the admixed populations every five years, and stacked barplots showing how the proportion of the three ancestries changed in said populations generation to generation as estimated by TRACTS, between the years 1500 and 2000 CE. Data on the number of slaves transported to each region, in log10 scale, are cumulative estimates of slaves disembarked there every 5 years based on records of trans-atlantic slave voyages from https://www.slavevoyages.org. Regions used are ports in North-Eastern South America for PEL & CLM, ports in what is now Mexico for MXL, ports on Spanish Carribean islands for PUR, ports north of the Rio Grande in North America for ASW, and ports on British Carribean islands for ACB. Genomic data of the individuals from each admixed population was analysed with TRACTS to 25 bootstraps, with generations being estimated as 25-year periods.

continuous ancestry tracts than would be expected under HWE.

Each homozygous continuous ancestry tract length distribution has a left tail absent in their haplotype counterparts, likely an artefact of heterozygous alleles breaking large homozygous tracts which would leave one of the two haplotype ones intact.

A more sophisticated software for continuous ancestry tract length analysis is TRACTS, which uses them to infer how many generations ago migration events took place. Cross-referencing this with relevent slave migration data provides a picture of delays between migration and significant admixture, ie assortative mating (**Fig. 6**). As it was Europeans transporting slaves accross the Atlantic, we know Europeans arrived in the region the same generation Africans began to arrive or earlier.

Therefore, for example in Peru, we can see that Europeans and Africans began arriving around 1525, the majority of Africans had arrived by 1575, significant admixture between Europeans and Natives occurred around 1650, and significant admixture between Africans and the rest of the population occurred around 1675. This suggests extreme assortative mating for 4-6 generations in Europeans and a similar length, albeit lagging by a generation, in Africans.

While the Mexican and Colombian plots can be interpretted similarly, the other three seem to suggest that the most significant African admixture occurred prior to 80-95% of the slaves being transported to the region, and spuriously that Europeans arrived at Barbados long before evidence suggests.

4 Discussion

Before drawing conclusions from analyses, the validity of the underlying data must be questioned. ASW is the code for Americans of Sub-Saharan African Ancestry from Oklahoma in Southwest USA, while MXL signifies Mexican Ancestry in Los Angeles California, which have significant sample biases. The ASW samples will likely have more african than the average US Southwest resident, and MXL samples more European and possibly African ancestry than the average Mexican.

The RFMIX reference panel was heavily imbalanced, with 72 Native samples to 507 European and 550 African. This few Native samples will make the algorithm more likely to assign SNP aleles to European or African despite being more indicative of Native ancestry. This could be responsible for the assignment of aproximately 5% European ancestry in the mayan population (**Fig. 1**), and can be resolved by sequencing more Native American genomes. Also, based on **Fig. S2**, ADMIXTURE seems to estimate 100% African or 0% European more readily than RFMIX, suggesting it may be less sensitive at those two extremes. Hence RFMIX ancestry proportion estimates might have been the better choice to go forward with, but an instumental systematic error like this is unlikely to significantly impact subsequent analysis.

One aim of this project was to test the hypothesis that the levels of assortative mating in the studied populations were both significant, and significantly different to each other. This hypothesis was supported in full by the results of the AMI analysis, both with triallelic and anestry-specific AMI (**Fig. 3-4**). The other aim was to assess the methods used throughout, including this AMI analysis, by their ability to differentiate assortative mating from migration, and thus whether the results are valid: a far more nuanced task.

In theory, Hardy-Weinberg equilibrium is established in a population following a single generation of fully random admixture (Smithjohn et al., 2015). This means two generations after large-scale migration, a population without ancestry-related social stratification should exhibit insignificant levels of assortative mating. Given all query populations exhibited significant levels, if we assume none of the samples are from first-generation immigrants then we can conclude that not only are the AMI analysis results valid, but that the method successfully distinguises between assortative mating and migration. It also highlights HWE as inapropriate as a concept for quality-checking genetic markers in genome-wide association studies, for which it it still widely used (Linares-Pineda et al., 2012), owing to the non-random admixture in human populations demostrated herein contradicting the assumptions upon which HWE relies (Smithjohn et al., 2015).

However, the AMI analysis method only allows us to reach that conclusion for the present-day populations, it tells us little about their past. Continuous ancestry tract analysis is better-suited for this, but the results are less conclusive. The histogram visualisation of the homozygous tract analysis (**Fig. 5B**) not only indicates that the right skewness in the **Fig. 5A** African ASW and

ACB plots and Native PEL plot are due to migration rather than assortative mating, but also shows in the Native MXL distribution that two peaks can mascerade as one. This method does not quantify the length of time passed since admixture, and hence can only be used to highlight major same-ancestry migration events in the past for further investigation: it fails to differentiate minor and prolonged migration from assortative mating.

The method integrating TRACTS with slave voyage data does inform us about the past, by quantifying time since admixture. The **Fig. 6** plots for the Peruvian, Mexican and Colombian populations are intuitive and historically plausible, although more detailed research into whether documentation of the period corroborates the projections should be conducted. Perhaps relatedly, these are the three query populations for which the slave voyages data used was most geographically accurate.

In the others, the majority of the slaves were purportedly transported after the generation of most significant admixture, roughly by an order of magnitude in all three cases. More thoroughly researching the history of trans-Atlantic slavery of these three regions and thus more accurately determining the ports at which slaves that ended up in Barbados, Puerto Rico and the US Southwest initially disembarked from their voyage may bring them more into line with the others. Specifically for the Barbados plot, even with the concept of an initital Native population hard-coded into the model, the algorithm could only explain the genomic pattern by predicting that Europeans arrived 50-100 generations ago, 500 or more years before it really occured. This is likely because ADMIXTURE estimated that only 2 out of the 96 samples contain any Native DNA, both with a proportion of less than 0.1 - a stark reminder that assortative mating and migration weren't the only population-shaping phenomena at play in the colonial-era Americas.

These are not the only issues with this TRACTS analysis. The analysis uses ancestry proportion, not absolute quantity of genetic material. This means Native populations shrinking due to disease and other consequences of colonialism would have the same effect of increasing European ancestry proportion in the population as European migration. Additionally, when interpretting TRACTS plots it must be remembered that TRACTS is constrained to only one pulse per ancestry, at the generation it deems to have had the biggest effect on the proportion of that ancestry. Until a superior software permits predicted ancestry proportion to change each generation, comparing TRACTS output with migration data has limited utility. Likewise, using African migration timing as a proxy for European migration rather than also integrating European migration data will limit the potential of the method.

Finally, an inherent flaw in analysing social stratification using generation as the unit of time - albeit unavoidable in genetic research - is that generation length is likely to differ significantly by subgroup, and indeed over time. For example, in slave-based societies of the southern US and carribean, slaves breeding was a cheaper method of procuring additional slaves, hence one might expect African subpopulations to have had shorter generation lengths earlier on in the Americas. In a truly stratified society, one would expect different stratas to have different generation lengths.

Ultimately, migration and the halting of assortative mating in the past, both the removal of barriers to admixture, manifest themselves near-identically. Therefore, the two are seemingly inextricable when projecting into the past absent accurate migration data to explain the contribution of migration to admixture, thereby leaving only assortative mating. However, it may be possible to use artificial neural networks to circumvent this need for migration data. Firstly, a model to

predict continuous ancestry tract length distribution based on input parameters such as level of assortative mating must be created. This model can be used to simulate tract length distriutions with every combination of input parameters. An artificial neural network can then be trained to learn the patterns between these distriutions and the corresponding paramters. In theory, it may subsequently be able to accurately predict the parameters, including level of assortative mating in the population, when applied to the tract length distributions generated in this study with empirical data - artificial neural networks have been successfully trained in this way before (Sheehan & Song, 2016).

The AMI analysis, having been established as a legitimate technique for distinguishing between migration and assortative mating, could be used to keep track of ancestry-related social stratification. As genome sequencing gets cheaper, larger and more selected sample sizes will enable more reliable results. Samples could be taken from those in small age windows in incraments of say 10 years to get a picture of such stratification in a population for the past few generations, and samples could be taken from young people every 10 years going forward to keep track of it in the long-term, perhaps to inform governmental policy.

While artificial neural networks have potential in bypassing the issue of inadequate migration data, integrating migration data into the simulation model would make the method even more powerful. This may not be possible with the current records of migration in the colonial-era Americas, but could be used in modern populations: much higher-quality migration data is available, although globalisation is leading to increasing ancestral diversity in populations, and accounting for more ancestries adds complexity. Like the AMI analysis, this could have promise in keeping track of ancestry-related social stratification in modern populations.

To increase the accuracy of these methods in quantifying assortative mating, another factor must be considered. Admixture of two ancestries may seem antithetical to ancestry-related social stratification, but not all admixture in a population is mutually voluntary. Many instances of admixture between slavemaster and slave, or colonist and Native, were involuntary and thus symptomatic of social stratification. To prevent involuntary admixture from counteracting assortative mating as a proxy for ancestry-related social stratification, this would ideally be quantified and integrated into the model. If it were assumed that negligable involuntary admixture occurred between European Females and Native or African Males, similar analyses but with the recombining section of the X chromosome rather than autosomes could be conducted. Discrepencies between assortative mating in European female to non-European male admixture and in European male to non-European female admixture could shed light on this phenomenon, as could sex-specific contributions from each ancestry in each population (Micheletti et al., 2020).

Assortative mating has long been neglected as a factor influencing admixture, whether in research into the the effects of migration on a population's genome or in genetic marker selection for genome-wide association studies. By improving upon and developing the methods utilised and suggested in this paper, powerful tools for the estimation of past and present assortative mating may be possible. Not only would this allow us to correct for assortative mating in the aforementioned studies, but it would enable us to better understand the history of human societies and may even enable us to monitor and thus combat present-day ancestry-related social stratification.

5 Data and Code Availability

5.1 Data

1KGP Samples:
https://www.internationalgenome.org/data-portal/data-collection/30x-grch38

HGDP Samples:
https://www.internationalgenome.org/data-portal/data-collection/hgdp

Phasing Reference Panel:
http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data_collections/1000G_2504_high_coverage/working/20201028_3202_phased/

Phasing Genetic Map:
https://github.com/odelaneau/shapeit4/blob/master/maps/genetic_maps.b38.tar.gz

Slave Voyage Data:
https://www.slavevoyages.org/voyage/database#tables (see tracts_mig_plots.R for details)

5.2 Code

Code Repository:
https://github.com/Bennouhan/cmeecoursework/tree/master/project/code

A detailed visualisation of the project's workflow can be found in **Fig. S6**, indicating which script(s) were used during each step in the analyses. See the README.md for further details.

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Supplementary Material

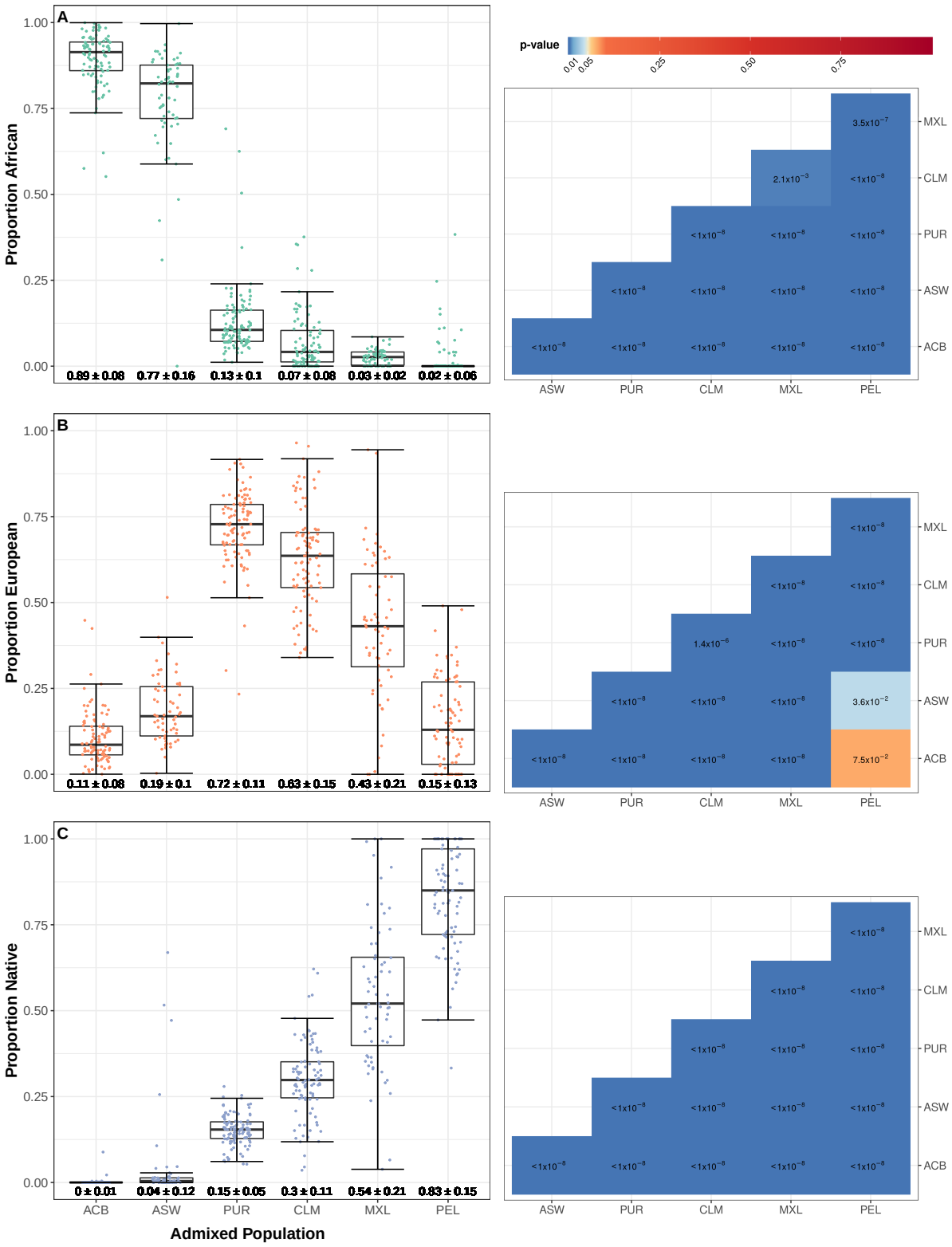

Figure S1: Comparative box plots displaying the distributions of the three ancestry proportions for each individual of each admixed population, with corresponding p-value heatmaps comparing populations statistically. The boxes highlight the upper quartile, median and lower quartile values of the distribtuion, while the whiskers signify the last data point within the closest quartile value plus 150% of the interquartile range. Mean ± standard deviation is given beneath. Horizontal jitter is used to better display the distribution. To the right of the boxplots for each ancestry is a corresponding p-value heatmap. These show the results of wilcoxon tests conducted between every combination of two admixed populations, with shades of blue indicating differences between populations are significant at the 5% level.

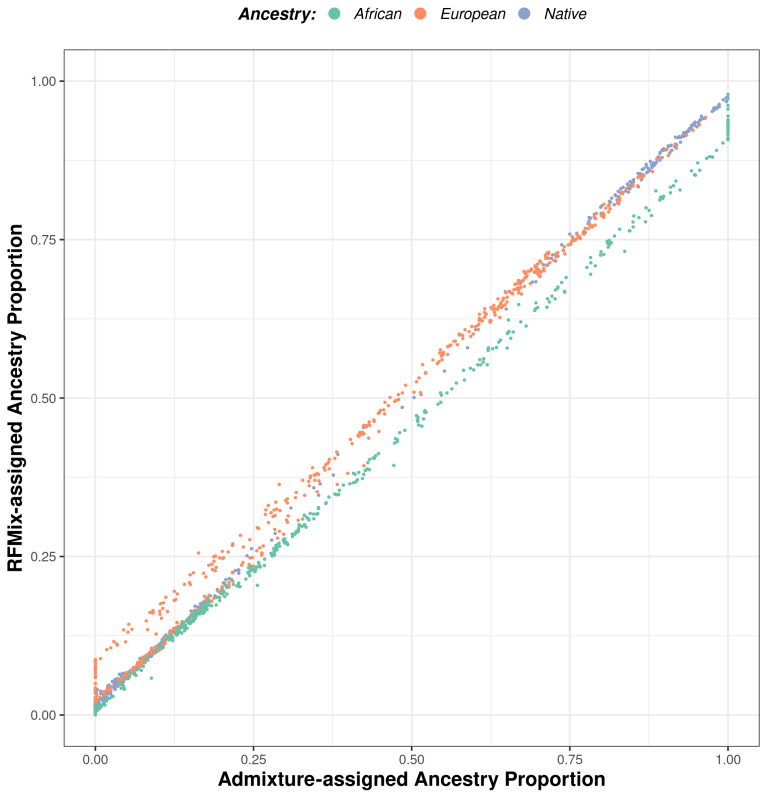

Figure S2: Scatterplot correlating ancestry proportions assigned by RFMIX for all 1690 query and reference individuals against those assigned by ADMIXTURE.

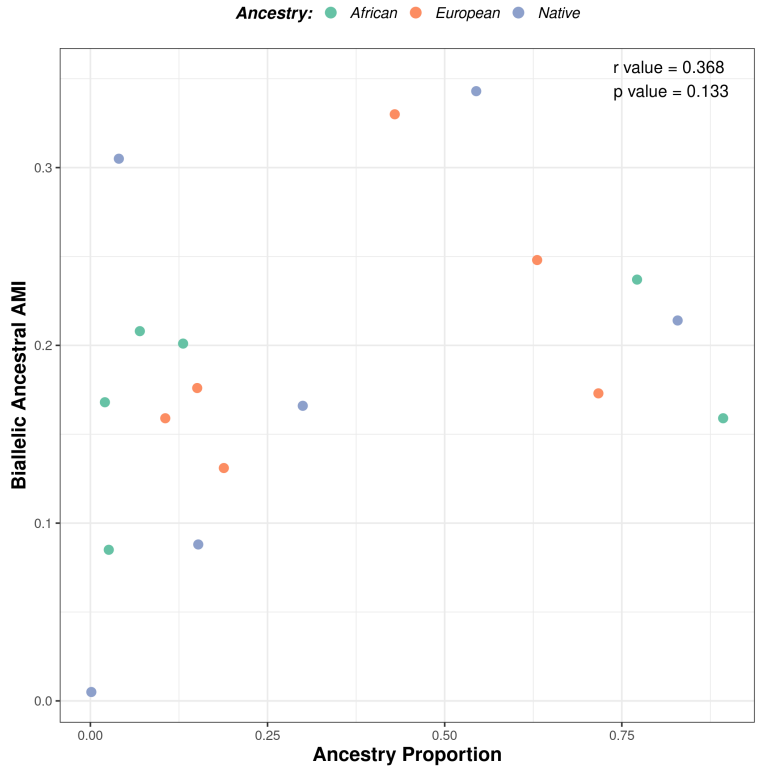

Figure S3: Scatterplot charting all three mean biallelic ancestry-specific AMI against all three ancestry proportion for each of the six admixed populations.

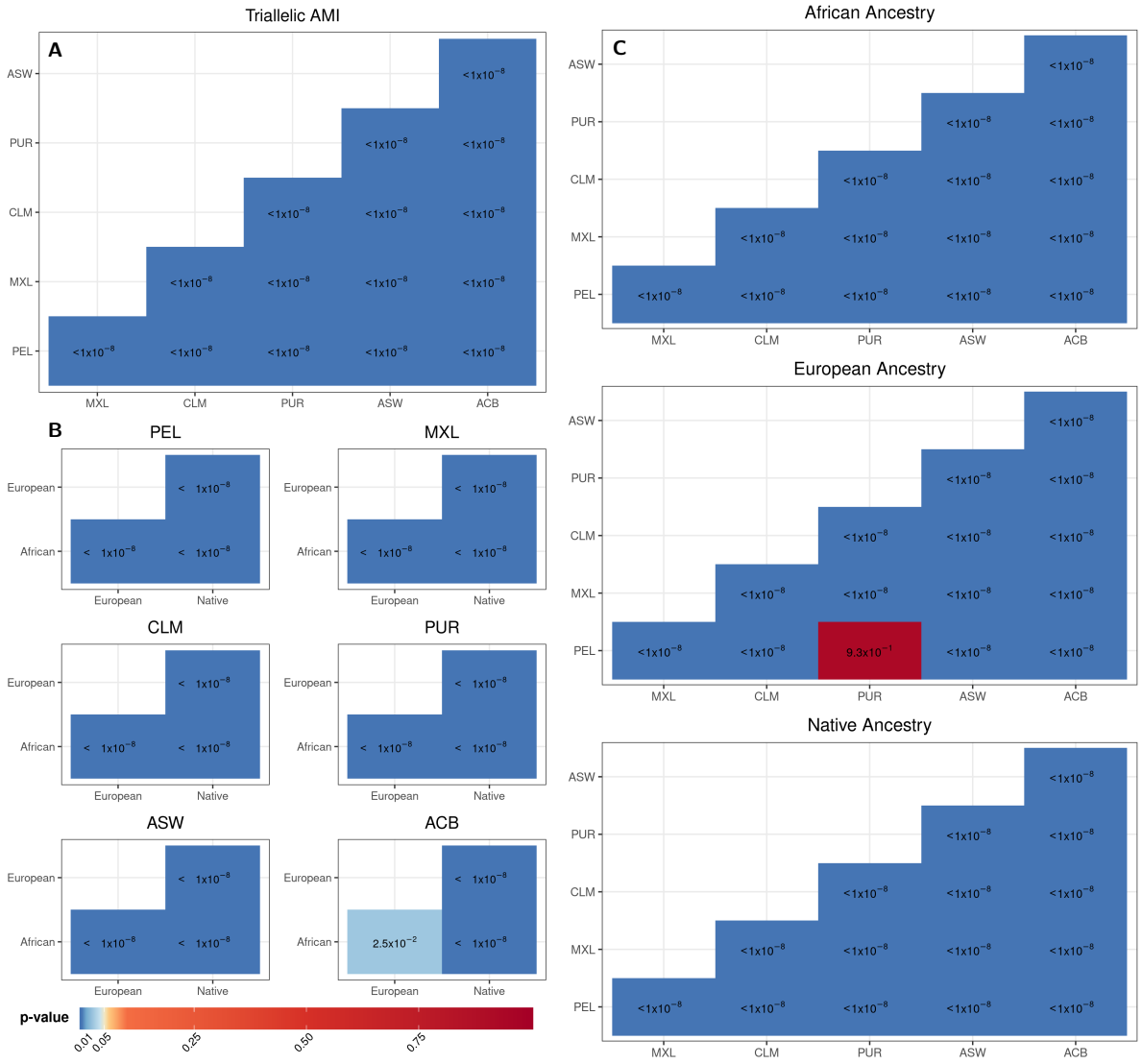

Figure S4: Heatmaps displaying p-value results of Wilcoxon tests used to compare assortative mating index values of different populations and ancestries. Each set of heatmaps correspond to a different set of comparisons between all combinations of assortative mating index (AMI) distributions. **A** compares all combinations of the six admixed populations with regards to their triallelic AMI distriutions, shown in **Fig. 3**. **B** compares all combinations of the three ancestries with regards to their biallelic ancestry-specific AMI distriutions, for each of the six admixed populations. **C** compares all combinations of the six admixed populations with regards to their biallelic ancestry-specific AMI distriutions, for each of the three ancestries, shown in **Fig. 4A-C**. Shades of blue indicate differences between populations or ancestries are significant at the 5% level.

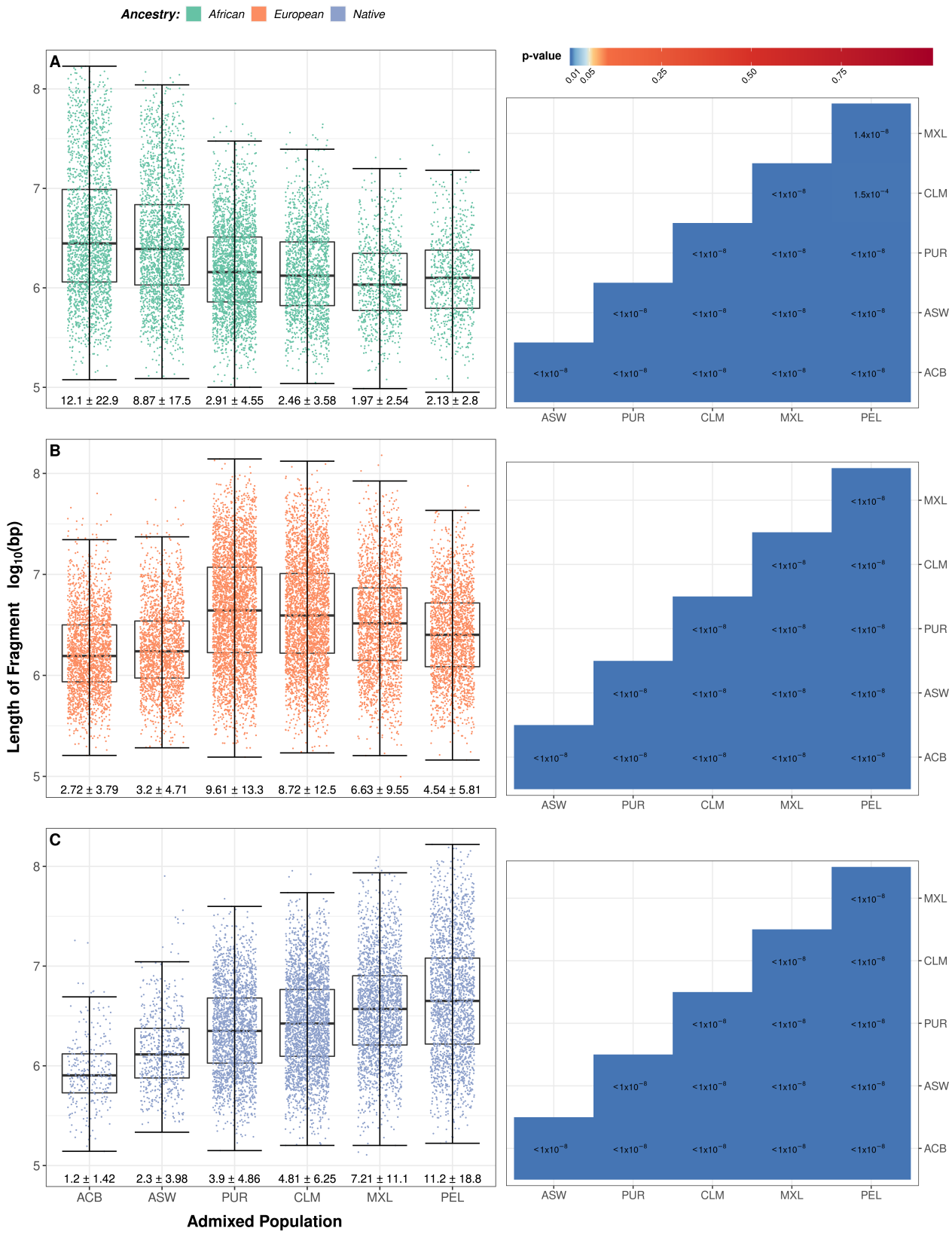

Figure S5: Comparative box plots displaying the distributions of continuous ancestry tract lengths of each ancestry for all individuals of each admixed population, with corresponding p-value heatmaps comparing populations statistically. Fragment length, that is the number of consecutive haplotype asignments of a given ancestry on a single strand, are measured in base pairs in log10 scale. The boxes highlight the upper quartile, median and lower quartile values of the distribtuion, while the whiskers signify the last data point within the closest quartile value plus 150% of the interquartile range. Mean ± standard deviation is given beneath in units of Mbp. Horizontal jitter is used to better display the distribution. To the right of the boxplots for Afrinca, European and Native ancestries (**A-C**) is a corresponding p-value heatmap. These show the results of wilcoxon tests conducted between every combination of two admixed populations, with shades of blue indicating differences between populations are significant at the 5% level.

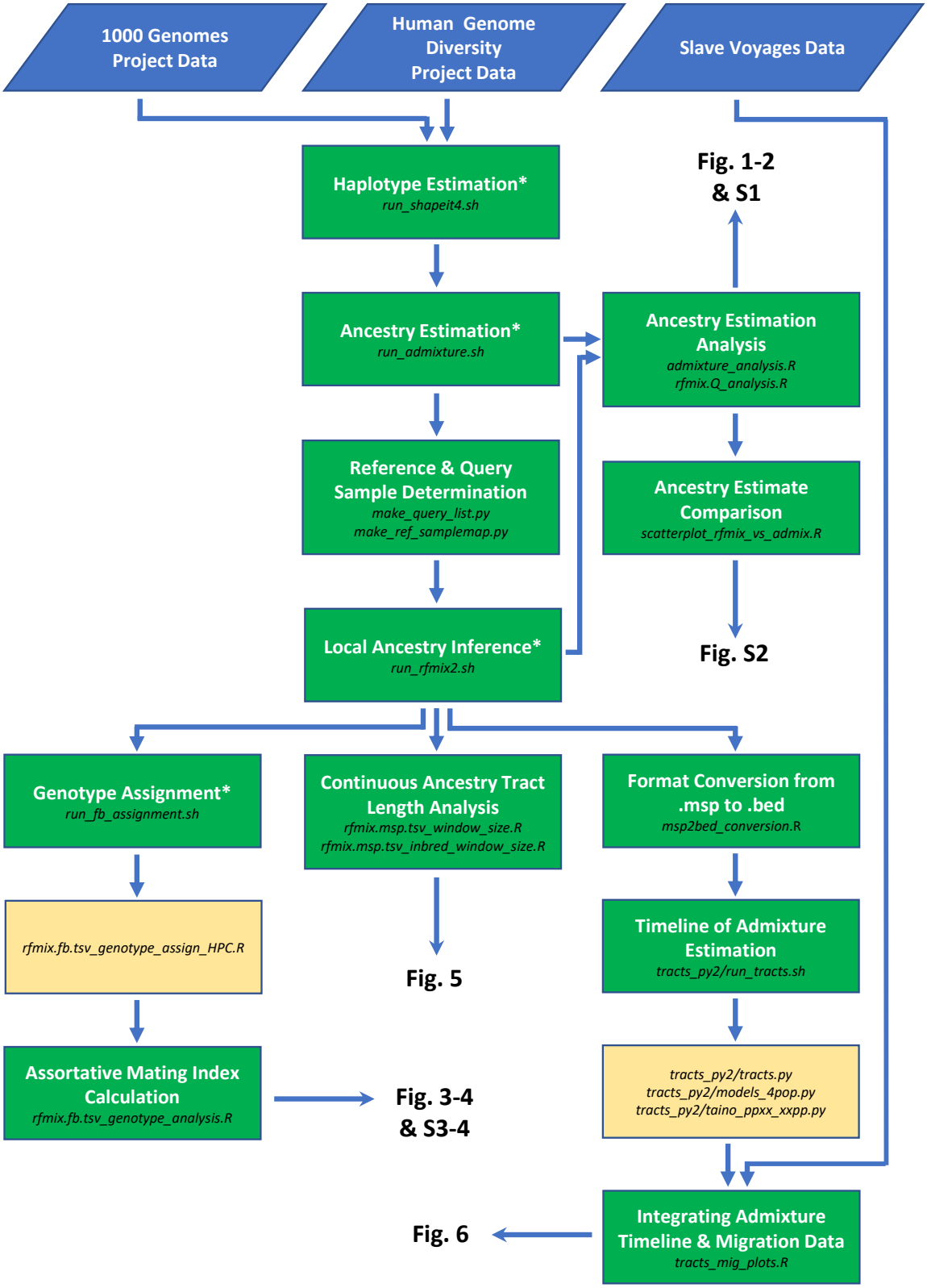

Figure S6: Flowchart representing the analysis workflow of the project, from input data to the output figures. Arrows indicate that the output from one step is the input for the next. Below the label of each step is the script(s) from the provided github repository required to run that step. The scripts named in the unlabelled yellow boxes are run automatically by the script in the previous step. Asterisked step labels indicate this step was performed on a high-performance computer due to the computational power required.