

Local PCA Shows How Population Structure Differs Along the Genome

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July 6, 2016

Abstract

Dimension reduction techniques, such as principal component analysis (PCA), are often used to discover and display large-scale structure in genomic datasets found in the patterns of kinship between the genotyped individuals, and to control for the confounding effects of population structure in genome-wide association studies. The genome-wide mean kinship that principal component uses is an average of the relationships across all locus-specific genealogical trees. However, many biological factors, including linked selection, can systematically skew patterns of kinship over intermediate genomic scales. We show how to use principal components analysis (PCA) to describe this meso-scale variation in kinship, and apply the method to genomic data from three species. In each species we find that population structure varies on the scale of megabases to tens of megabases. In a global human dataset, small, discontinuous variation is likely explained by polymorphic chromosomal inversions. In a dataset of primarily African *Drosophila melanogaster*, large, continuous variation across each chromosome arm is explained by known chromosomal inversions thought to be under recent selection. In a range-wide dataset of *Medicago truncatula*, common axes of variation in population structure are shared between chromosomes, correlate with local gene density, and may be caused by background selection or local adap-

tation. The method is a useful addition to the exploratory toolbox of population genomics, and is implemented as an R package.

Add percent of variance explained by first two MDS coordinates, after subtracting the genome-wide mean covariance matrix.

Add references to supplemental whole-genome PCA plots in the text.

1 Introduction

The phrase “population structure” refers to reduced gene flow between subpopulations, often because of geographical isolation. However, it is widely recognized that because of selection the effects of gene flow are not equal everywhere on the genome, and patterns of polymorphism and divergence can vary significantly depending on factors including local gene density. This implies that, paradoxically, the population structure of a species depends on which part of the genome is being examined.

Population structure leads to systematic patterns in genome-wide mean kinship, and so it is said that visualizations of kinship depict population structure, rather than the *effects* of population structure (which would be more accurate). The *kinship coefficient* for a pair of individuals gives the expected proportion of their genome that the two have inherited identically by descent; for a single individual it is the inbreeding coefficient. However, as Wright (1949) wrote, “It has probably occurred to the reader that the coefficient of inbreeding may mean very different things in different cases.” The kinship coefficient originally referred to the expected probability of coinheritance within a given pedigree; while modern applications to “unrelated” individuals use a genetic covariance matrix to estimate the *realized* proportion of the genome coinherited from sufficiently recent ancestors.

Realized kinship summarizes the shapes of the genealogical trees that relate the samples at each location along the genome. Since these trees vary along the genome, so does

realized kinship; but averaging over sufficiently many trees we hope to get a stable estimate, independent of the genomic region chosen. This hope is not entirely justified: for instance, kinship on sex chromosomes is expected to differ from the autosomes; and positive or negative selection on particular loci can dramatically distort shapes of nearby genealogies (Barton 2000; Charlesworth 2012; Maynard Smith and Haigh 1974; Neher 2013), and therefore patterns of kinship. Indeed, chromosome-scale variation in diversity and divergence has been observed in many species (e.g. Langley et al. 2012); species phylogenies can differ along the genome due to incomplete lineage sorting (e.g. Pease and Hahn 2013), adaptive introgression and/or local adaptation (e.g. Ellegren et al. 2012; Nadeau et al. 2012; Pool 2015; Vernot and Akey 2014); and theoretical expectations predict that geographic patterns of relatedness should depend on selection (Charlesworth et al. 2003). Nonetheless, it is not generally known to what extent patterns of kinship vary along the genome, nor what the major axes of variation are.

Patterns in genome-wide kinship are often summarized by applying principal components analysis (PCA) (Patterson et al. 2006) to the genetic covariance matrix, as pioneered by Menozzi et al. (1978). The results of PCA can be directly related to the underlying genealogical history of the samples, such as time to most recent common ancestor and migration rate between populations (McVean 2009; Novembre and Stephens 2008). These patterns are often geographical, producing “maps” of population structure that reflect the samples’ geographic origin distorted by rates of gene flow (Novembre et al. 2008), although other patterns emerge if recent migration or nongeographic kinship patterns are having a strong role in relatedness determining. (Astle and Balding 2009).

As reviewed by Astle and Balding (2009), modeling “background” kinship between samples is essential to successful genome-wide association studies, and PCA has often been used for stratification correction (Price et al. 2006), and so investigating variation

in kinship along the genome can help us to have a better understanding of the relation between genome structure and population structure, possibly leading to more powerful methods for association studies.

To investigate how population structure varies along the genome in several datasets, we cut each genome into windows (with hundreds to thousands of SNPs in each), applied PCA to each window, and visualized the major ways that population structure, as summarized by PCA, varies among windows. whole genome sequencing data for *Drosophila*. To quantify similarity of population structure between windows, we constructed for each window an approximate, scaled covariance matrix based on the first few principal components, and measured the pairwise Euclidean distance between those matrices. We then use multidimensional scaling to visualize the relationships between windows, which reduces the pairwise distance matrix to lower dimension while preserving the distance information between windows as well as possible (Borg and Groenen 2005).

Each species showed distinct patterns, reflecting differences in their biology; before presenting results, it may be helpful to have a prior idea of what factors are expected to affect population structure, and how. PCA summarizes patterns in kinship found in the genetic covariance matrix, which is an average across locus-specific genealogies. If individuals are closer in the genealogies of a given genomic region, they tend to be closer in the PCA maps for that region. Strong selective sweeps of beneficial alleles can lead to relatively long genomic regions characterized by short genealogical trees (Garud et al. 2013; Przeworski et al. 2005). A genomic region with many targets for selection experiences background selection and/or recurrent selective sweeps (Coop and Ralph 2012; Stephan et al. 1992), which would tend to shorten genealogical trees in the region, similar to a reduction in effective population size (Hudson and Kaplan 1995; Sattath et al. 2011). On the other hand, balancing selection leads to very deep trees (Gao et al. 2014), and population structure

locally describes which individuals have which alleles rather than geographical proximity. Finally, since recombination is suppressed between opposite orientations of a chromosomal inversion near its breakpoints, genealogies in these regions separate samples carrying the two orientations of the inversion. In the resulting extended block, population structure shows two (for haploids) or three (for diploids) clusters (indeed, (Ma and Amos 2012) has proposed using trimodality of local PCA plots as a way to identify inversions). We will look for strong variation in population structure shared across large regions of the genome. Many of the effects listed above (such as single selective sweeps or inversions) are not expected to have similar effects on population structure in different regions of the genome because of randomness in which samples end up in which group; but if these coincide with a region of reduced recombination (such as an inversion), these could drive major patterns of variation. The more subtle effects of genome-wide linked selection could be shared across large regions due to large-scale variation in gene density (as by background selection or local adaptation at many genes).

We chose PCA to summarize population structure, but other methods, such as STRUCTURE (Falush et al. 2003), SPA (Yang et al. 2012), SpaceMix (Bradburd et al. 2015), or other matrix factorization methods (Engelhardt and Stephens 2010) would highlight different sources of variation in the data.

Finally, a number of general methods for dimensionality reduction also use a strategy of “local PCA” (e.g. Kambhatla and Leen 1997; Manjón et al. 2013; Roweis and Saul 2000; Weingessel and Hornik 2000), performing PCA not on the entire dataset but instead on subsets of observations, providing “local” pictures which are then stitched back together to give a global picture. At first sight, this differs from our use of the term in that we restrict to subsets of *variables* instead of subsets of observations. However, if we flip perspectives and think of each genetic variant as an observation, our method shares common threads,

although the ultimate goals and methods for visualization are different. Future methods for visualization of genomic data may benefit from other advances in this substantial literature (reviewed in Van Der Maaten et al. 2009).

2 Methods

The general steps in our method could be carried out in many ways. Referring to Figure 1, these are (1) divide the genome into windows, (2) summarize the patterns of relatedness (population structure) in each window, (3) measure dissimilarity in population structure between each pair of windows, (4) visualize the resulting dissimilarity matrix, and (5) combine similar windows to more accurately estimate local population structure. Details of how we carried these out are given below.

2.1 Datasets

We used three publically available datasets (summarized in Table 1); as usual for genetic data we converted the data to a numeric matrix (with one row per polymorphic variant and one column per sample) by replacing each genotype with the number of nonreference alleles (or NA for missing data). A normalization step (see below) ensures the result does not depend on the choice of reference allele.

Human: We used genomic data from the entire POPRES dataset (Nelson et al. 2008), which has array-derived genotype information for 447,267 SNPs across the autosomes of 3,965 samples in total: 346 African-Americans, 73 Asians, 3,187 Europeans and 359 Indian Asians. (We excluded the sex chromosomes and the mitochondria.) We use the allele that has highest frequency in the samples as the reference allele for each position.

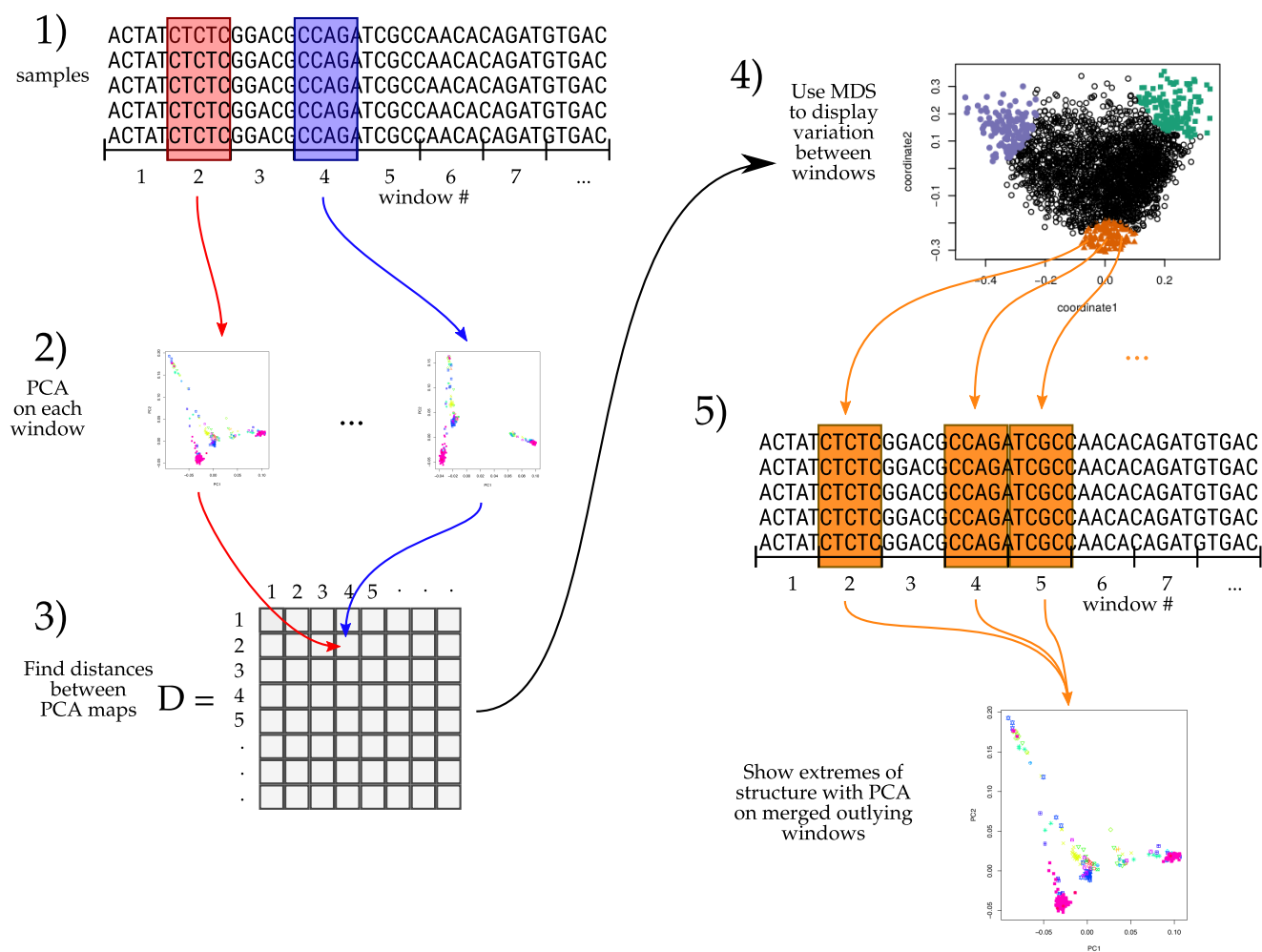


Figure 1: An illustration of the method; see text for details.

***Drosophila melanogaster*:** We also used whole-genome sequencing data from *Drosophila* Population Genomics Project (DPGP) (Lack et al. 2015), which together has 380 samples from 16 countries across Africa and Europe. Since the *Drosophila* samples are from inbred lines, we treat the samples as haploid when recoding; regions with residual heterozygosity were marked as missing in the original dataset. Each chromosome arm we investigated (Chr2L, Chr2R, Chr3L, and Chr3R) has 2–3 million SNPs. Due to high density of missing data for some parts in the genome, we first deleted any samples with more than 8% missing genotypes, and then deleted positions with more than 20% missing data. (We chose these cutoffs as the tails of the relevant empirical distributions.)

***Medicago truncatula*:** Finally, we used whole-genome sequencing data from the *Medicago truncatula* Hapmap Project (Tang et al. 2014), which has 263 samples from 24 countries, primarily distributed around the Mediterranean basin. Each of the 8 chromosomes has 3-5 million SNPs; we did not use the mitochondria or chloroplasts.

species	# SNPs per window	mean window length (bp)	mean # windows per chromosome	mean % variance explained by top 2 PCs
Human	100	636,494	203	0.55
<i>Drosophila melanogaster</i>	1,000	9,019	2,674	0.53
<i>Medicago truncatula</i>	10,000	102,580	467	0.50

Table 1: Descriptive statistics for each dataset used, with the chosen window sizes. See text for how window sizes were chosen; in each window we performed PCA, computed the percent variance explained by the top two principal components; the mean across windows is given in the last column.

2.2 PCA in genomic windows

After recoding, we divided the genome into contiguous segments (“windows”; see below), and applied Principal Component Analysis (PCA) as described in McVean (2009) sepa-

rately to the submatrices that corresponded to each window. Specifically, we did PCA as follows: denote by Z the $L \times N$ recoded genotype matrix (L is the number of SNPs and N is the sample size), and by \overline{Z}_s the mean of non-missing entries for allele s , so that $\overline{Z}_s = \frac{1}{n_s} \sum_j Z_{sj}$, where the sum is over the n_s nonmissing genotypes. We first compute the mean-centered matrix X , as $X_{si} = Z_{si} - \overline{Z}_s$, and preserving missingness. (This mean-centering makes the result not depend on the choice of reference allele, exactly if there is no missing data, and approximately otherwise.) Next, we find the covariance matrix of X , denoted C , as $C_{ij} = \frac{1}{m_{ij}-1} \sum_s X_{si} X_{sj} - \frac{1}{m_{ij}(m_{ij}-1)} (\sum_s X_{si})(\sum_s X_{sj})$, where all sums are over the m_{ij} sites where both sample i and sample j have nonmissing genotypes. The principal components are the eigenvectors of C , normalized to have Euclidean length equal to one, and ordered by magnitude of the eigenvalues.

The top few principal components generally display population structure; we usually use the first two (referred to as $PC1$ and $PC2$). The above procedure can be performed on any subset of the data; for future reference, denote by $PC1_j$ and $PC2_j$ the result after applying to all SNPs in the j^{th} window.

Since eigenvectors are still only defined up to sign, when comparing between windows we choose the sign to best match each other: after choosing $PC1_1$, for instance, and u is the first eigenvector obtained from the covariance matrix for window j , then $PC1_j = \pm u$, where the sign is chosen according to which of $\sum_i (PC1_{i1} - u_i)^2$ or $\sum_i (PC1_{i1} + u_i)^2$ is smaller.

2.3 Choosing window length

The choice of window length entails a balance between several factors. In very short windows, genealogies of the samples will only be represented by a few trees, so variation between windows represents demographic noise rather than meaningful variation in popula-

tion structure. Longer windows generally have more distinct trees (and SNPs), allowing for less noisy estimation of local population structure. However, to better resolve meaningful signal, i.e., differences in population structure along the genome, we would like reasonably short windows. Window length choice therefore entails a signal versus noise tradeoff in the estimates of population structure. Since we summarize population structure using relative positions in the principal component maps, we quantify “noise” as the standard error of a sample’s position on PC1 in a particular window, averaged across windows and samples, and “signal” as the standard deviation of the sample’s position on PC1 over all windows, averaged over samples. (Recall that the signs for PCs are chosen to match each other.) Then, the mean variance across windows is

$$\sigma_{\text{signal}}^2 = \frac{1}{N} \sum_{j=1}^N \frac{1}{L} \sum_{i=1}^L (PC1_{ij} - \overline{PC1}_j)^2,$$

where $\overline{PC1}_j = (1/N) \sum_{i=1}^N PC1_{ij}$. We estimate the standard error for each $PC1_{ij}$ using the block jackknife (Efron and Efron 1982): we divide the j^{th} block into 10 equal-sized pieces, and let $PC1_{ij,k}$ denote the first principal component of this region found after removing the k^{th} piece; then the estimate of the squared standard error is $\sigma_{ij}^2 = \frac{9}{10} \sum_{k=1}^{10} (PC1_{ij,k} - \frac{1}{10} \sum_{\ell=1}^{10} PC1_{ij,\ell})^2$, and we measure noise by averaging over samples and windows:

$$\sigma_{\text{noise}}^2 = \frac{1}{N} \sum_{j=1}^N \frac{1}{L} \sum_{i=1}^L \sigma_{ij}^2.$$

We calculated σ_{signal}^2 and σ_{noise}^2 for a range of window sizes, and for our main results chose the smallest window for which σ_{signal}^2 was consistently larger than σ_{noise}^2 ; the values for various window sizes across *Drosophila* chromosomes are shown in Table 2, and the choices for all taxa are in Table 1. In practice, even substantially different window sizes

result in the same genome-wide patterns. We also chose windows to each consist of the same number of neighboring SNPs. We compared to the results when windows are each of the same physical length (in bp) but of the same average length, and results were nearly identical.

chrom. arm		window length (SNPs)				
		100	500	1,000	10,000	100,000
2L	σ_{noise}^2	2.05	1.64	1.18	0.17	0.04
	σ_{signal}^2	2.76	2.69	2.23	0.68	0.31
2R	σ_{noise}^2	2.18	1.92	1.63	0.58	0.13
	σ_{signal}^2	2.78	2.70	2.65	2.31	1.82
3L	σ_{noise}^2	2.08	2.00	1.64	0.73	0.25
	σ_{signal}^2	2.60	2.52	2.40	1.68	1.89
3R	σ_{noise}^2	1.95	1.76	1.44	0.59	0.20
	σ_{signal}^2	2.58	2.51	2.44	1.96	1.40
X	σ_{noise}^2	2.48	2.04	1.54	1.62	0.17
	σ_{signal}^2	2.61	2.43	2.30	0.32	1.14

Table 2: Measures of signal and noise, computed separately for each chromosome arm in the *Drosophila* dataset, at different window sizes. All values are multiplied by 1,000 (so typical variation is of order of 50% of the actual values). Starting at windows of 1,000 SNPs, the signal (variation of PC1 between windows) starts to be substantially larger than the noise (standard error of PC1 for each window).

2.4 Similarity of population structure between windows

We think of local population structure as being summarized by *relative* position of the samples in the space defined by the top principal components. Since rotations of this space imply identical population structure, we do not compare population structure of different genomic windows using the PCs directly, but instead compare the low-dimensional approximations of the local covariance matrices obtained using the top k PCs. (For results shown here, we use $k = 2$; results using larger numbers of PCs were nearly identical.) We do this, rather than using the entire covariance matrix, both for computational efficiency

and because the top PCs summarize important population structure, so using only these should reduce the effect of noise. To remove the effect of variation in mutation rate between windows, we also rescale each covariance matrix so that the underlying data matrix has trace norm equal to one. To do this, define the $N \times k$ matrix $V(i)$ so that the ℓ^{th} column of $V(i)$ is equal to the ℓ^{th} principal component of the i^{th} window, multiplied by $\sqrt{\lambda_{\ell i} / \sum_{m=1}^k \lambda_{mi}}$. Then, the rescaled, k -dimensional covariance matrix for the i^{th} window is

$$M(i) = \sum_{\ell=1}^k V_{i\ell} V_{i\ell}^T. \quad (1)$$

We then use Euclidean distance D_{ij} between the matrices $M(i)$ and $M(j)$ to measure the similarity of population structure for the i^{th} window and j^{th} window. Since $\sum_{ij} (A_{ij} - B_{ij})^2 = \sum_{ij} (A_{ij}^2 + B_{ij}^2) - 2 \text{tr}(A^T B)$, and $M(i) = V(i) V(i)^T$, then due to the orthogonality of eigenvectors and the cyclic invariance of trace, D_{ij} can be computed efficiently as

$$D_{ij} = \frac{\sum_{\ell=1}^k \lambda_{\ell i}^2}{(\sum_{\ell=1}^k \lambda_{\ell i})^2} + \frac{\sum_{\ell=1}^k \lambda_{\ell j}^2}{(\sum_{\ell=1}^k \lambda_{\ell j})^2} - 2 \sum_{\ell,m=1}^k (V(i)^T V(j))_{\ell m}^2. \quad (2)$$

2.5 Visualization of results

We use multidimensional scaling (MDS) to visualize relationships between windows as summarized by the dissimilarity matrix D , that produces a set of m coordinates for each window that give the arrangement in m -dimensional space that best recapitulates the original distance matrix. For results here, we use $m = 2$ to produce one- or two-dimensional visualizations of relationships between windows' population structure.

We then locate variation in population structure along the genome by choosing collections of windows that are nearby in MDS coordinates, and map their positions along the

genome. Their common population structure is identified by extracting the corresponding genomic regions and performing PCA on all, aggregated regions.

2.6 Software

The methods described here are implemented in an open-source R package available at <https://github.com/petrelharp/lostruct>, as well as scripts to perform all analyses from VCF files at various parameter settings.

3 Results

In these three species, PCA plots vary along the genome in a systematic way, showing strong chromosome-scale correlations. This implies that variation is due to meaningful variation in population structure, since fluctuations due to demographic noise are not expected to show long distance correlations. Below, we discuss the results and likely underlying causes.

3.1 *Drosophila melanogaster*

We ran the method on chromosome arms 2L, 2R, 3L, 3R and X separately. For each, the two-dimensional MDS visualization resembles a triangle, as seen in Figure 2. Since the relative position of each window in this plot shows the similarity between windows, this suggests that there are at least three extreme types of population structure typified by windows found in the three corners of the triangle, and that other windows’ population structure may be a mixture of those extremes.

To investigate these extremes, for each chromosome arm we visually selected three “extreme” windows in the MDS plot, and take out the 5% of windows that are closest to it in the MDS coordinates; these three groups of windows are shown in color in Figure 2 and PCA plots for each collection are shown in Figure 3.

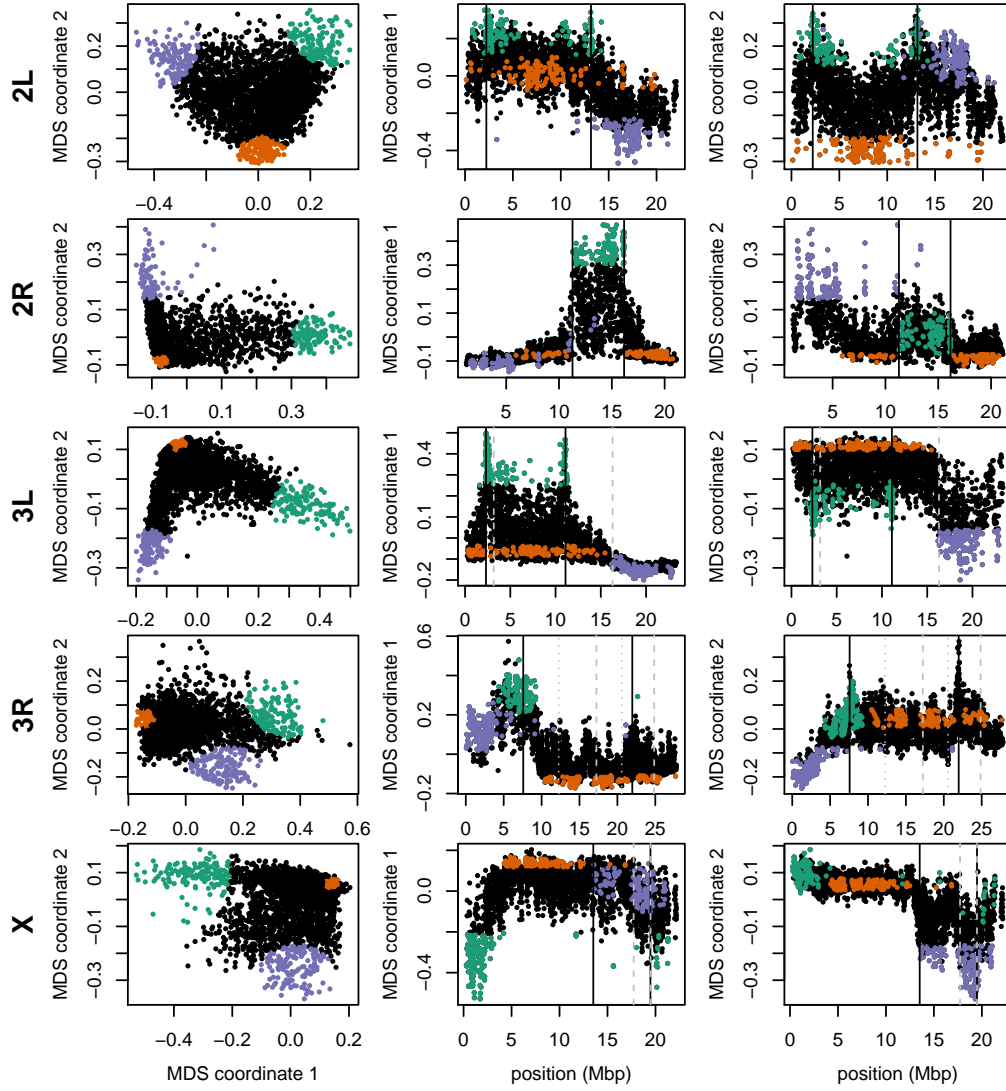


Figure 2: Variation in structure for windows across *Drosophila melanogaster* chromosome arms. In all plots, each point represents one window along the genome. The first column shows the MDS visualization of relationships between windows, and the second and third columns show the midpoint of each window against the two MDS coordinates; rows correspond to chromosome arms. Colors are consistent for plots in each row. Vertical lines show the breakpoints of known polymorphic inversions.

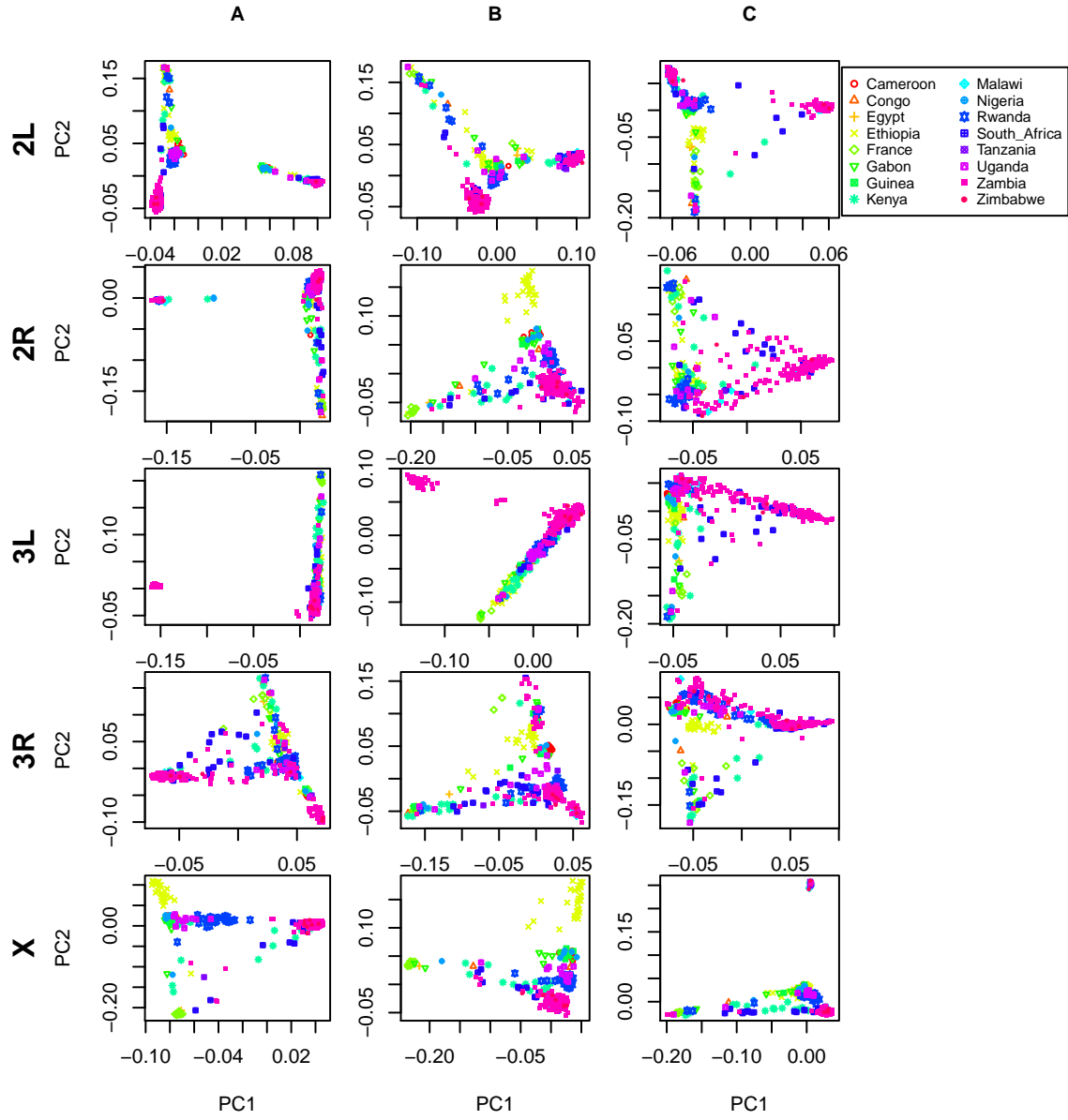


Figure 3: PCA plots for the three sets of genomic windows colored in Figure 2, on each chromosome arm of *Drosophila melanogaster*. In all plots, each point represents a sample. The first column shows the combined PCA plot for windows whose points are colored green in Figure 2; the second is for orange windows; and third is for purple windows.

The variation in population structure turns out to be almost entirely explained by several large inversions that are polymorphic in these samples, identified in Corbett-Detig and Hartl (2012). We recolored the PCA plots in Figure 3 by the orientation of the inversion for each sample, obtaining Figure 4. Taking chromosome arm 2L as an example, the two regions of similar, extreme population structure shown in green in the first row of Figure 2 lie directly around the breakpoints of the inversion In(2L)t, and the PCA plots in the first rows of Figure 4 shows that population structure here is mostly determined by inversion orientation. The regions shown in purple on chromosome 2L lie near the centromere, and have population structure reflective of two axes of variation, seen in Figure 3, which correspond roughly to latitude within Africa and to degree of cosmopolitan admixture respectively (see Pool (2015) for more details). *check that reference* The regions shown in orange on chromosome 2L mostly lie inside the inversion, and show population structure that is a mixture between the other two, as expected due to recombination within the (long) inversion (Kirkpatrick and Barrett 2015). *check that reference too* Similar results are found in other chromosome arms, except that the situation of Chr3R is a little more complicated, due to the coexistence of two polymorphic inversions (In(3R)K and In(3R)P) at intermediate frequency. However, peaks in MDS coordinates along the chromosome show the breakpoints of both, although we have not highlighted portions of the MDS plot that pick these out. Two more inversions, In(3L)P and In(3R)Mo, are at very low frequency in these samples, and do not significantly influence population structure.

3.2 Human

As for the *Drosophila* data, we ran our method separately on all 22 human autosomes, with results shown in Supplementary Figure 11, 12, 13. On each, variation in population structure was dominated by a small number of windows having similar population structure

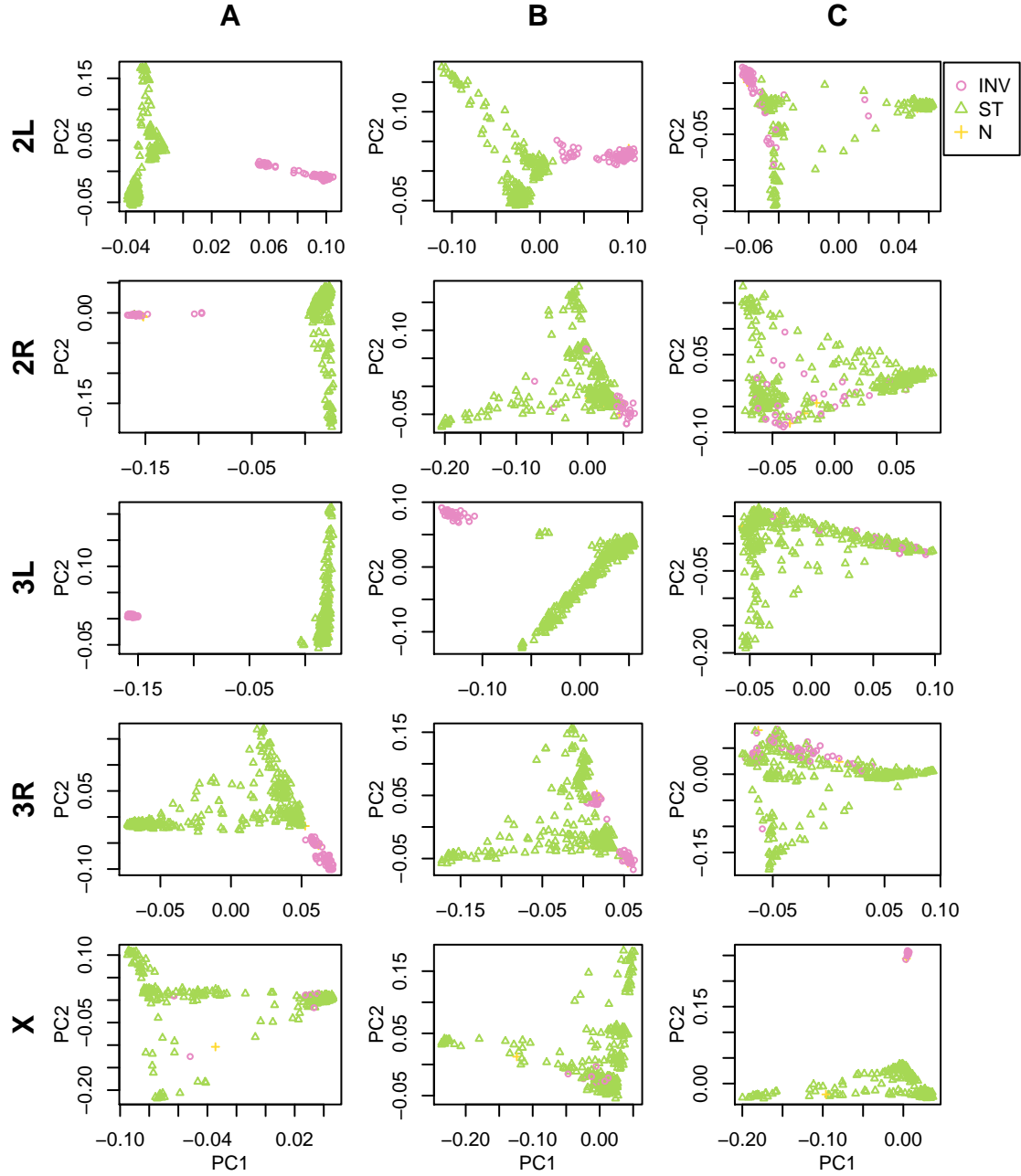


Figure 4: As in Figure 2, except that samples are colored by orientation of the corresponding polymorphic inversions, In(2L)t, In(2R)NS, In(3L)OK, In(3R)K and In(1)A respectively. (Data from Lack et al. (2015)).

to each other that differed dramatically from the rest of the chromosome. These may be primarily inversions: for instance, the eleven windows that are outliers in the first MDS coordinate of chromosome 8 (Figure 5) coincide with the position of a particularly large, polymorphic inversion on 8p23. More generally, we see signals coincident with three of the six large polymorphic inversions described in Antonacci et al. (2009), shown in Figure 5. PCA plots of these windows show a characteristic trimodal shape (Figure ??), presumably distinguishing samples having each of the three diploid genotypes for each inversion orientation (although we do not have data on orientation status), a signal that has been proposed as a method to identify inversions (Ma and Amos 2012). However, distinguishing this hypothesis from others, such as regions of low recombination rate, would require additional data.

We also ran the method on all 22 autosomes together, and found that, remarkably, the inversion on chromosome 8 is still the most striking outlying signal (Figure 10). Further investigation with a denser set of SNPs, allowing a finer genomic resolution, may yield other patterns.

3.3 *Medicago truncatula*

Unlike the other two species, the method applied separately on all eight chromosomes of *Medicago truncatula* showed similar patterns of gradual change in population structure across each chromosome, with no indications of chromosome-specific patterns. This consistency suggests that the factor driving the population structure for each chromosome is the same, as might be caused by varying strengths of linked selection. To verify that variation in population structure is shared across chromosomes, we applied the method to all chromosomes together. Results for chromosome 3 are shown in Figures 7 and 8, and other chromosomes are similar: the two types of population structure with higher gene

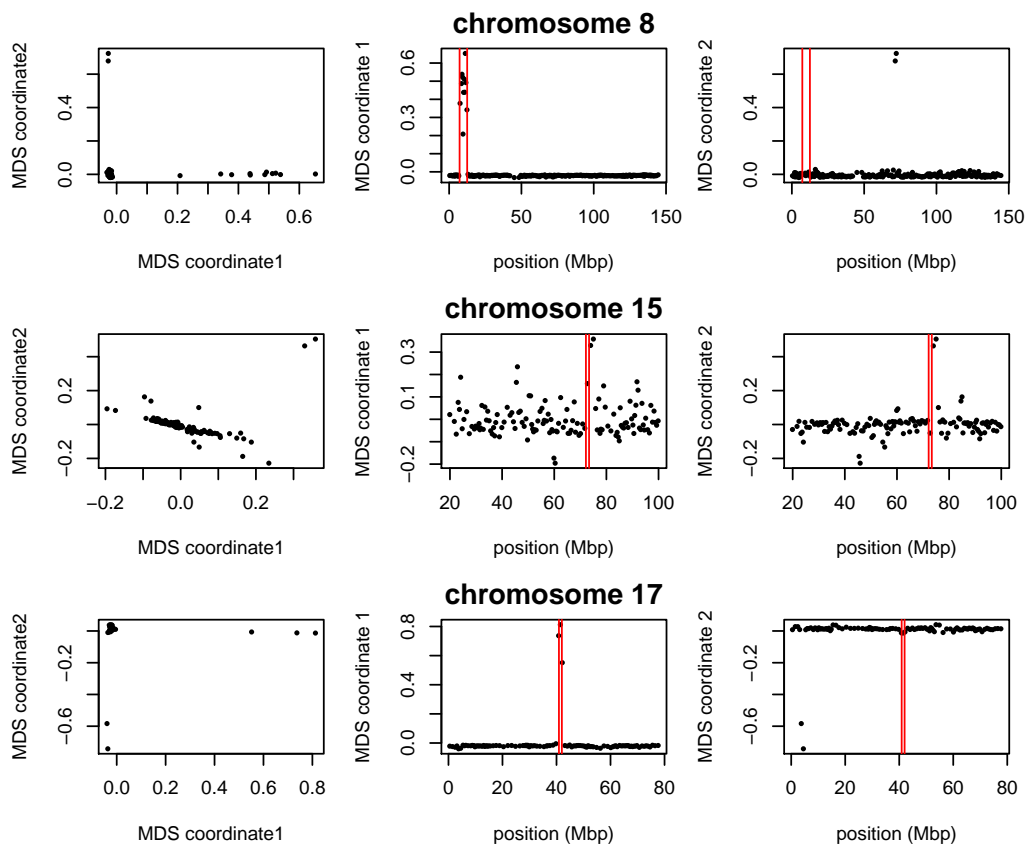


Figure 5: Variation in structure between windows on human chromosomes 8, 15, and 17. Each point in each plot represents a window. The first column shows the MDS visualization of relationships between windows; the second and third columns show the two MDS coordinates of each window against its position (midpoint) along the chromosome. Rows, from top to bottom show chromosomes 8, 15, and 17. The vertical red lines show the breakpoints of known inversions from (Antonacci et al. 2009).

density (colored green and purple in Figure 7 are nearly identical across all chromosomes, but population structure associated with low gene density (colored orange) shows more chromosome-specific patterns (see Supplemental Figure 17).

Across chromosomes, the high values of MDS coordinate 1 coincide with the position of the heterochromatic regions surrounding the centromere, which often have lower gene density and may therefore be less subject to linked selection (Kulikova et al. 2001; Paape et al. 2013). To verify that this is a possible explanation, we computed gene density for each window using gene models in Mt4.0 JBrowse (Tang et al. 2014). we downloaded the gene model track from Mt4.0 JBrowse at jcvi.org, extracted the start and end positions of each predicted mRNA transcript, counted how many of these fell in each window, and use the mean number of the starts and ends in a window to represent the gene count for that window. The endpoints of the windows were defined to be halfway between the last SNP of each window and the first SNP in the next window. These values are shown juxtaposed with the first MDS coordinate of each window is shown in Figure 6, and are significantly correlated, as shown in Figure 9. Other genomic features covary with gene density, however, so we cannot rule out alternative hypotheses.

We also found nearly identical results when choosing shorter windows of 1,000 SNPs; or choosing windows of equal length in base pairs rather than SNPs.

4 Discussion

Our investigations have found a remarkable amount of variation in population structure across the genomes of three diverse species, revealing distinct biological processes driving this variation in each species. More investigation, particularly on more species and datasets, will help to uncover which patterns are generalizable.

With growing appreciation of the heterogeneous effects of selection across the genome,

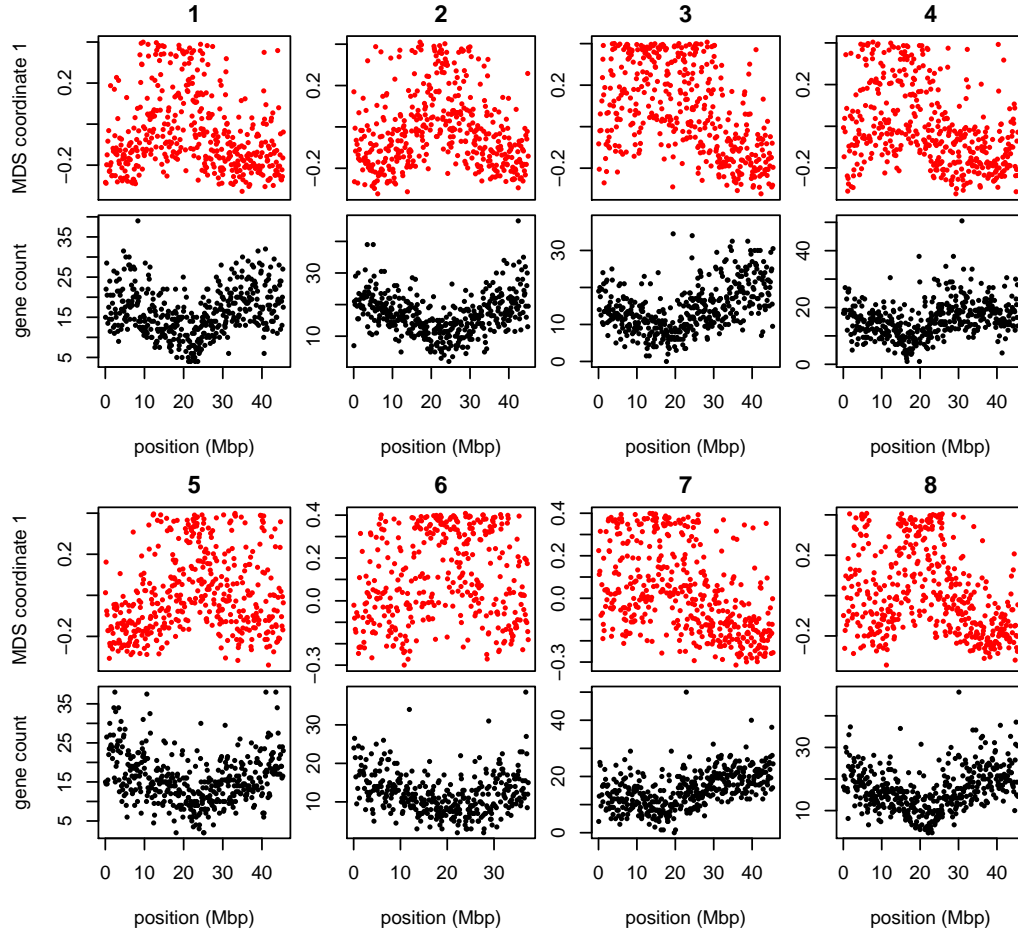


Figure 6: MDS visualization and gene density for each window in the *Medicago* genome, for chromosomes 1–8 (numbered above each pair of figures). For each chromosome, the red plot above is first coordinate of MDS against the middle position of each window along each chromosome. The black plot below is gene count for each window against the position of each window.

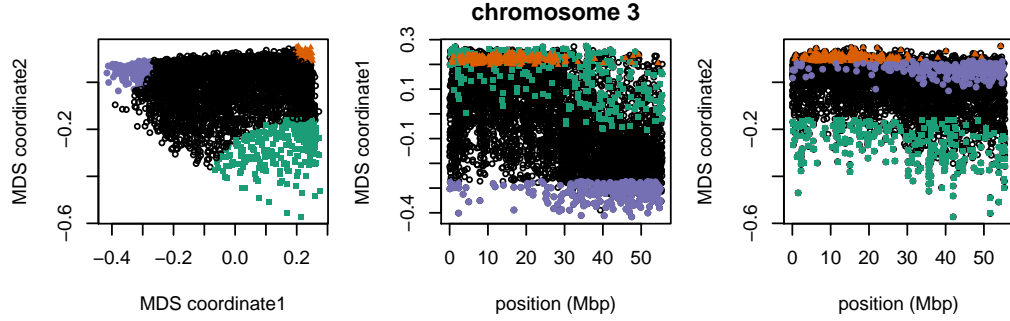


Figure 7: MDS visualization for *Medicago* chromosome 3. Each point in the plot represents a window. Plots for remaining chromosomes are shown in Supplemental Figure 15.

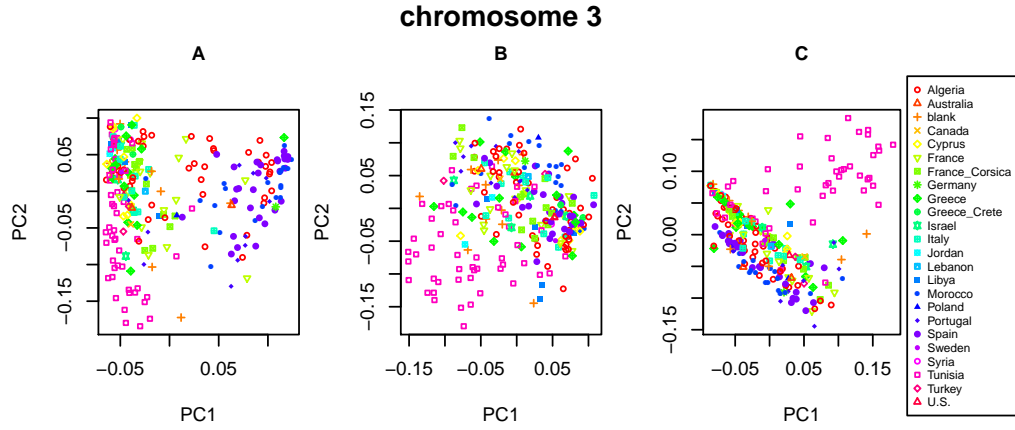


Figure 8: PCA plots for the sets of genomic windows colored (A) green, (B) orange, and (C) purple in Figure 7. Each point corresponds to a sample, colored by country of origin. Plots for remaining chromosomes are shown in Supplemental Figure 16.

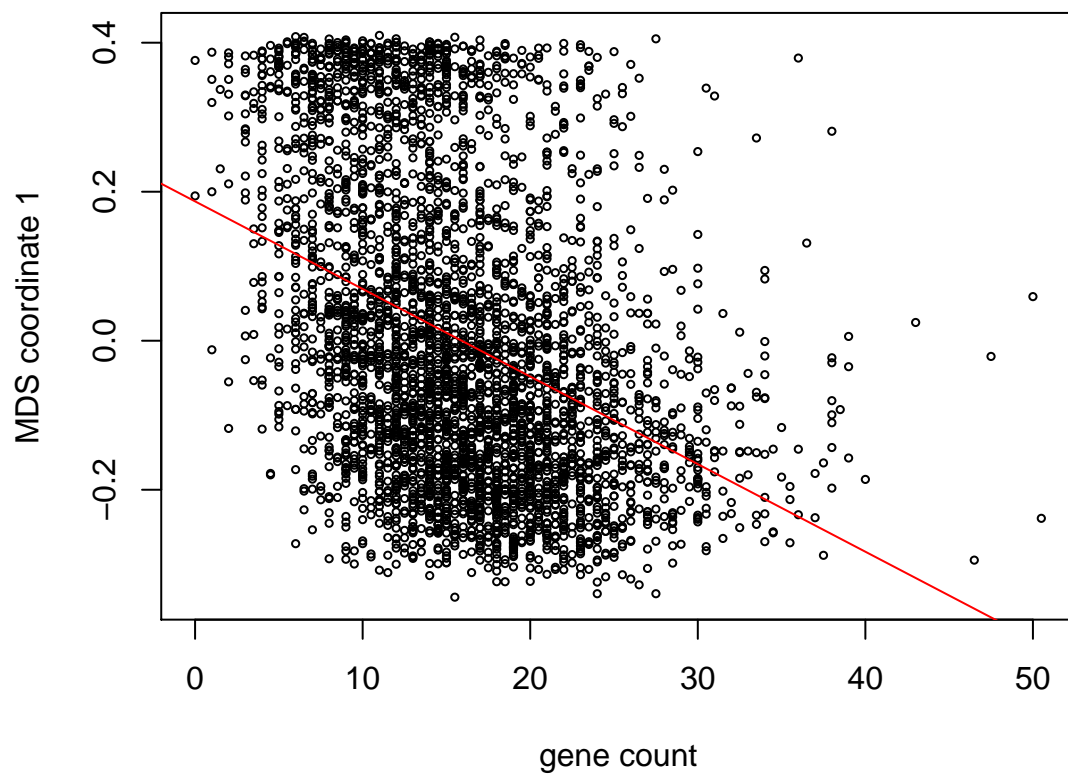


Figure 9: First MDS coordinate against gene density for all 8 chromosomes. The first MDS coordinate is significantly correlated with gene count ($r = 0.149$, $p = 2.2 \times 10^{-16}$). (See supplementary for each single chromosome's MDS result against gene count for *Medicago*)

especially the importance of adaptive introgression, hybrid speciation (Brandvain et al. 2014; Fitzpatrick et al. 2010; Hufford et al. 2013; Pool 2015; Staubach et al. 2012), local adaptation (Lenormand 2002; Wang and Bradburd 2014), and inversion polymorphisms (Kirkpatrick 2010; Kirkpatrick and Barrett 2015), local PCA may prove to be a useful exploratory tool to discover important genomic features. It is unclear whether the technique will be useful on reduced representation genotyping datasets due to marker density and issues with missing data – our investigations with one such dataset were inconclusive – but even low coverage, whole-genome sequence is very promising.

Confounding in GWAS So-called cryptic relatedness between samples has been one of the major sources of confounding in genome-wide association studies (GWAS) and so methods must account for it by modeling population structure or kinship (??). Since population structure is not constant along the genome, this could in principle lead to an inflation of false positives in parts of the genome with stronger population structure than the genome-wide average. Fortunately, in our human dataset this does not seem likely to have a strong effect: most variation is due to small, independent regions, possibly primarily inversions, and so may not have a major effect on GWAS. In the other species we examined, particularly *Drosophila melanogaster*, treating population structure as a single quantity could be severely misleading.

Parameter choices There are several choices in the method that may affect the results. As with whole-genome PCA, the choice of samples is important, as variation not strongly represented in the sample will not be discovered. The effects of strongly imbalanced sampling schemes are often corrected by dropping samples in overrepresented groups; but downweighting may be a better option that does not discard data. Next, the choice of window size may be important, although in our applications results were not sensitive

to this, indicating that we can see variation on a sufficiently fine scale. Finally, which collections of genomic regions are compared to each other (steps 3 and 4 in Figure 1), along with the method used to discover common structure, will affect results. We used MDS, applied to either each chromosome separately or to the entire genome; for instance, human inversions are clearly visible as outliers when compared to the rest of their chromosome, but genome-wide, their signal is obscured by the numerous other signals of comparable strength.

Besides window length, there is also the question of how to choose windows. In these applications we have used nonoverlapping windows with equal numbers of polymorphic sites. Alternatively, windows could be chosen to have equal length in genetic distance, so that each would have roughly the same amount of phylogenetic information. However, given the insensitivity of our results to window length, this seems unlikely to give different results.

More generally, there are many possible methods to discover common structure in different parts of the genome. The methods we chose discovered strong biological signal of different types in three datasets; but it is possible that other methods for measuring dissimilarity between windows' covariance matrices or for summarizing the matrix of pairwise distances between windows would lead to different insights. Minor points we have not explored include how to decide how many PCs to use in approximating structure of each window (equations 1), how many MDS coordinates to use when describing the distance matrix between windows, or how to choose interesting regions of the genome when the MDS plot is not triangular. These are all part of more general techniques in dimension reduction and high-dimensional data visualization; we encourage the user to experiment.

Chromosomal inversions A major driver of variation in population structure in two datasets we examined are inversions. This may be common, but the example of *Medicago*

truncatula shows that polymorphic inversions are not ubiquitous. PCA has been proposed as a method for discovering inversions (Ma and Amos 2012); However, the signal left by inversions likely cannot be distinguished from long haplotypes under balancing selection or simply regions of reduced recombination. However, in many applications, inversions are a nuisance. For instance, SMARTPCA (Patterson et al. 2006) reduces their effect on PCA plots by regressing out the effect of linked SNPs on each other. It would be interesting to see if somehow removing the effects of inversions in the *Drosophila melanogaster* or human datasets would produce a pattern similar to that seen in *Medicago truncatula*.

Acknowledgements

We are indebted to John Pool, Russ Corbett-Detig, Matilde Cordeiro, and Peter Chang for assistance with obtaining data and interpreting results (especially inversion status of *D. melanogaster* samples). Thanks also go to Yaniv Brandvain and Barbara Engelhardt for helpful comments and for encouraging the project. *Others?*

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A Weighted PCA

PCA can be thought of as finding a good low-dimensional matrix factorization (Engelhardt and Stephens 2010) that well-approximates the original data in the least-squares sense: if C is the $N \times N$ matrix of genotypes, then to find the top k principal components, we find an orthogonal $N \times k$ matrix U , and a $k \times k$ diagonal matrix Λ with diagonal entries $\Lambda_{ii} = \lambda_i$

to minimize

$$\|C - U\Lambda U^T\|^2 = \sum_{ij} \left(G_{ij} - \sum_m \lambda_m U_{im} U_{jm} \right)^2. \quad (3)$$

The columns of U , known as the principal components, are the eigenvectors of C , the eigenvalues of C are λ , and the proportion of variance explained by the m^{th} component is

$$\frac{\lambda_m^2}{\sum_{\ell} \lambda_{\ell}^2} = \frac{\sum_{ij} (\lambda_m U_{im} U_{jm})^2}{\sum_{ij} C_{ij}^2}.$$

Thinking about the problem as a least-squares approximation problem makes it clear why unbalanced sample sizes can result in undesirable outcomes. If we want to describe variation *between* populations, but 80% of the samples are from a single population, then unless populations are highly differentiated, a better approximation to C may be obtained by using the columns of U to describe variation *within* the overrepresented population rather than between the populations. A common workaround is to remove samples, but a more elegant solution can be found by reweighting the objective function in (3). Let w_i be a weight associated with sample i , W the diagonal matrix with w along the diagonal, and instead seek to minimize

$$\|W^{1/2}(C - U\Lambda U^T)W^{1/2}\|^2 = \sum_{ij} W_i W_j \left(G_{ij} - \sum_m \lambda_m U_{im} U_{jm} \right)^2, \quad (4)$$

and now for convenience we require U to be orthogonal in $\ell_2(w)$, i.e., that $U^T W U = I$. We then would choose w to give roughly equal weight to each *population*, instead of each individual. We have used with good results the weightings $w_i = 1/\max(10, n_i)$, where n_i is, if there are discrete populations, the number of samples in the same population as sample i ; or, for continuously sampled individuals, the number of samples within a certain

distance of sample i .

To solve (4), let λ and V denote the eigenvalues and eigenvectors of $W^{1/2}CW^{1/2}$, so that $V\Lambda V^T$ is the closest in least squares to $W^{1/2}CW^{1/2}$; so if we define $U = W^{-1/2}V$ then $U^T W U = V^T V = I$, and

$$W^{-1/2}V\Lambda V^T W^{-1/2} = U\Lambda U^T$$

is the low-dimensional approximation to C . The proportion of variance explained is calculated from eigenvalues as before, but has the interpretation

$$\frac{\lambda_m^2}{\sum_{\ell} \lambda_{\ell}^2} = \frac{\sum_{ij} w_i w_j (\lambda_m U_{im} U_{jm})^2}{\sum_{ij} w_i w_j C_{ij}^2}.$$

Note finally that since we only need find the top k eigenvectors; in our R implementation we use the Spectra library (Qiu and Mei 2016).

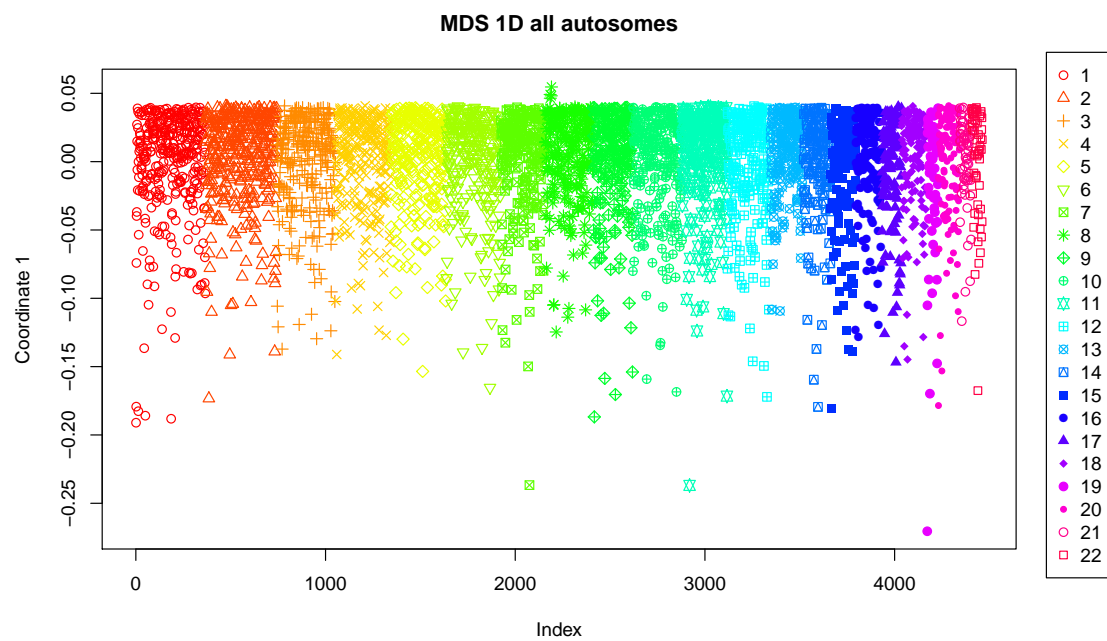


Figure 10: MDS 1D plot along all 22 autosomes in Human.

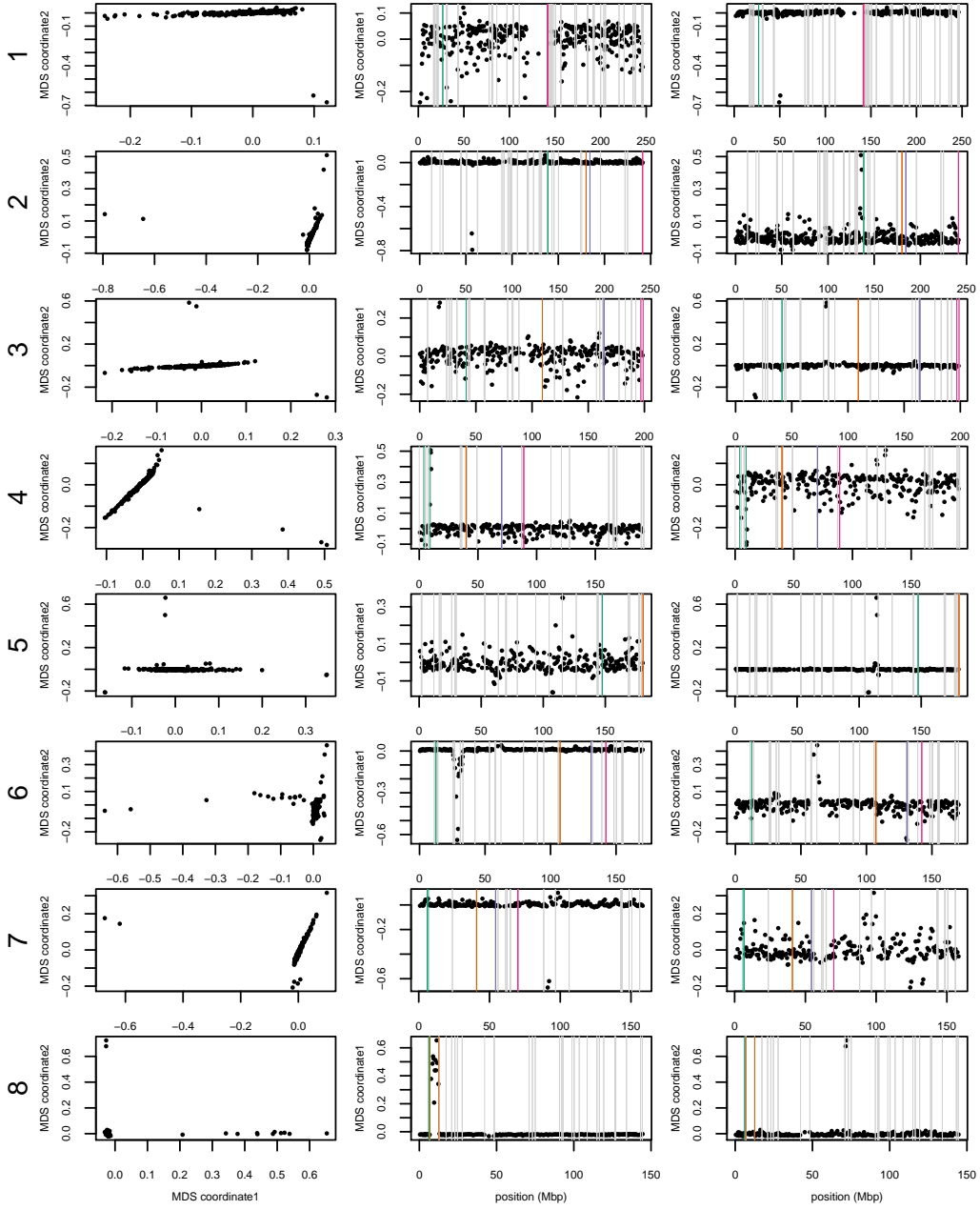


Figure 11: MDS plots for human chromosome 1-8. The first column shows the MDS visualization of relationships between windows, and the second and third columns show the midpoint of each window against the two MDS coordinates; rows correspond to chromosomes. Colorful vertical lines show the breakpoints of known valid inversions, while grey vertical lines show the breakpoints of predicted inversions.

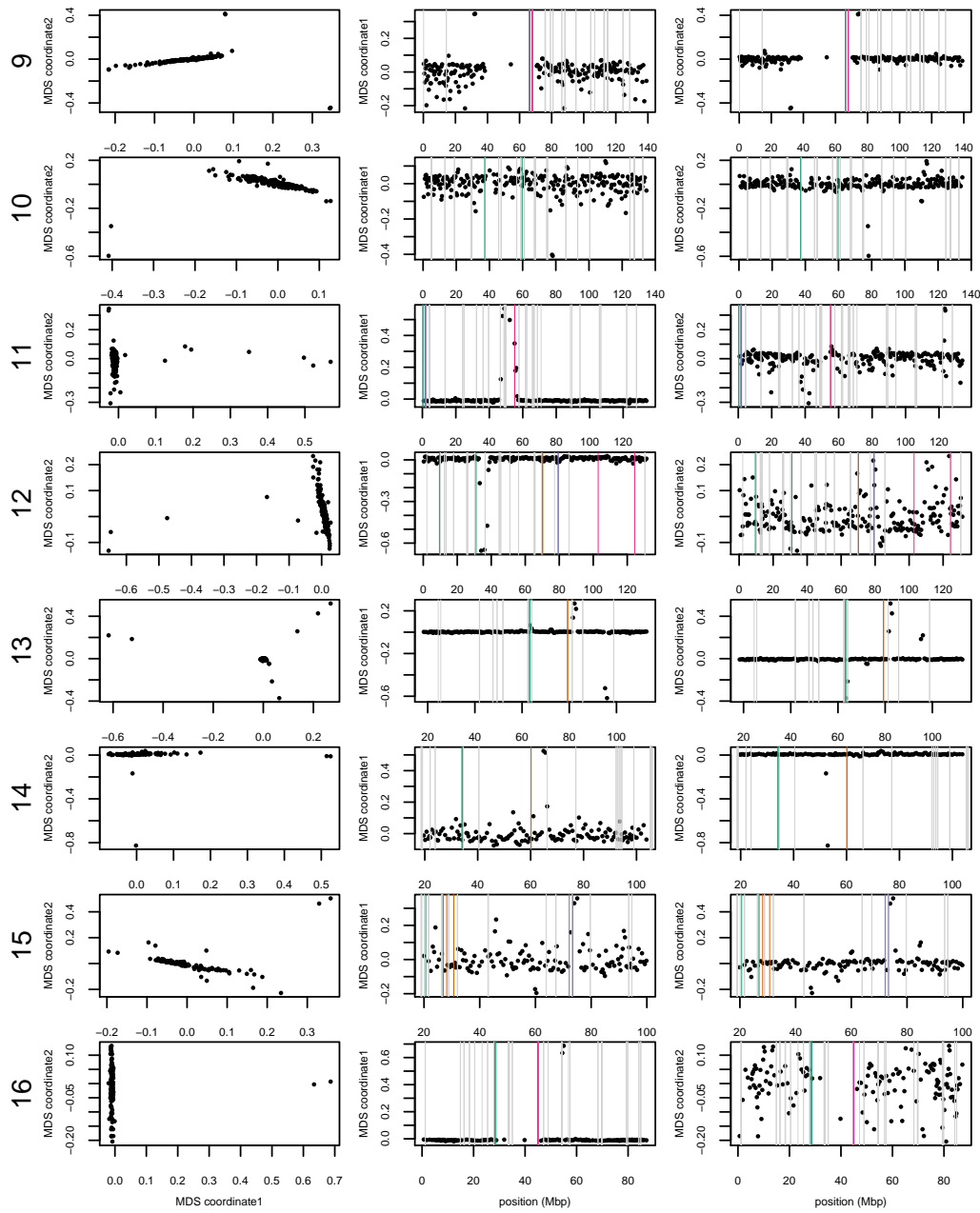


Figure 12: MDS plots for human chromosome 9-16.

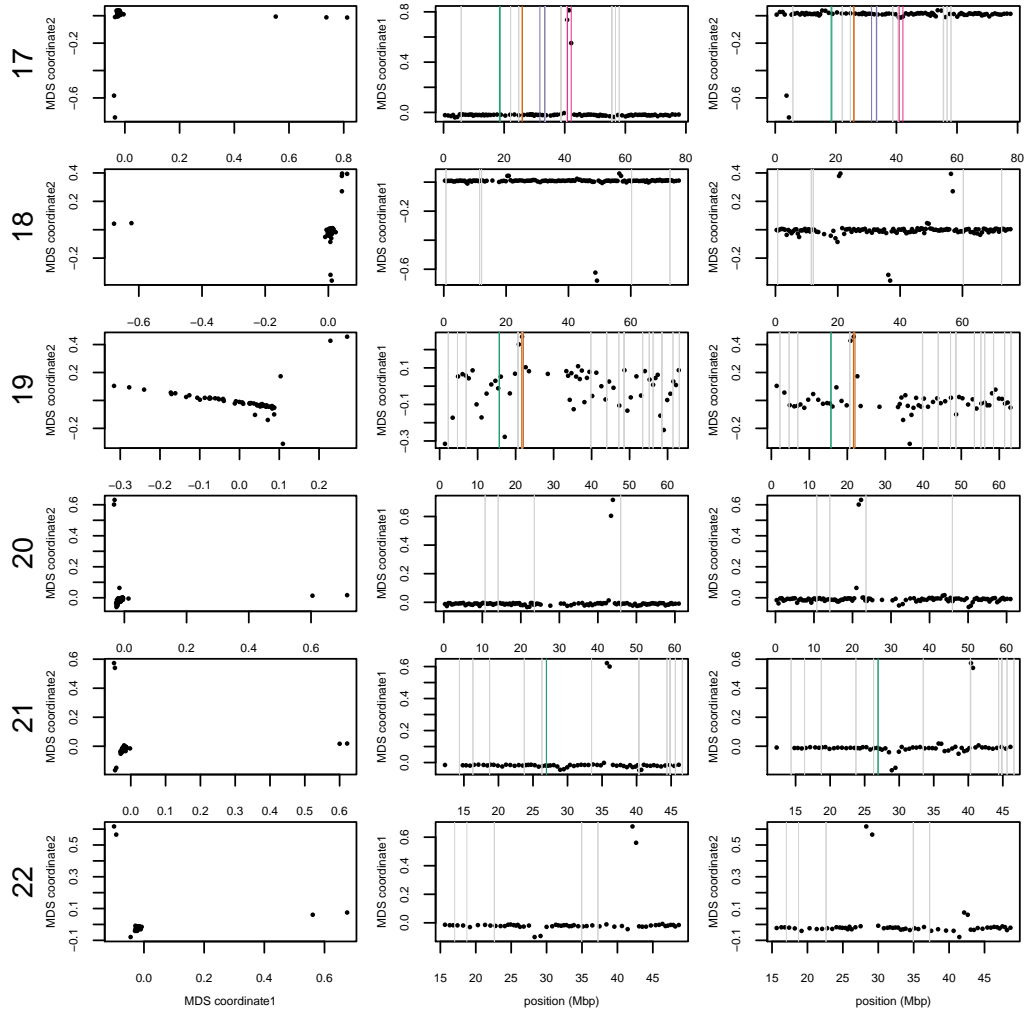


Figure 13: MDS plots for human chromosome 17-22.

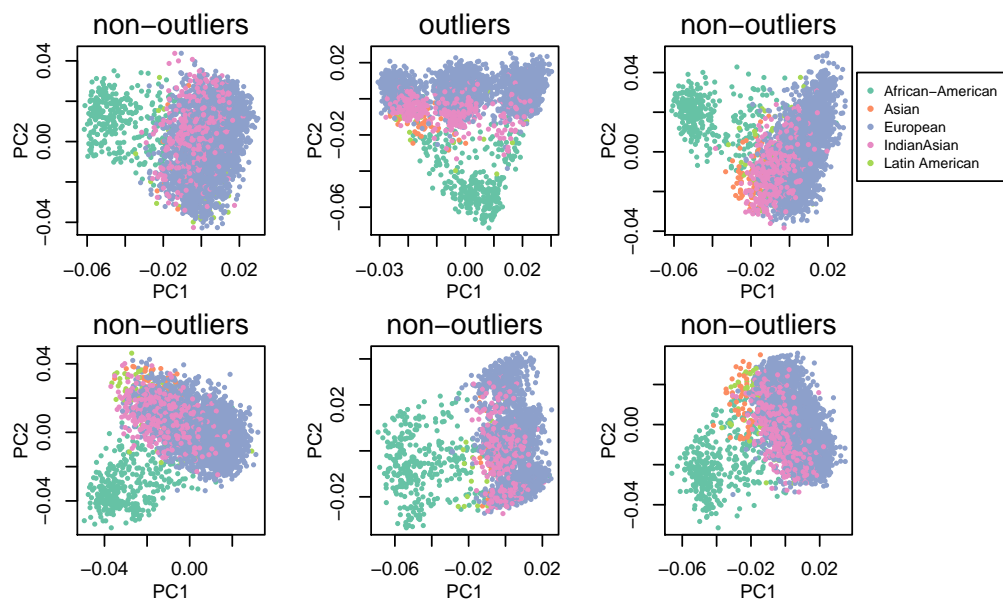


Figure 14: PCA plots comparison for outlier windows and non-outlier windows

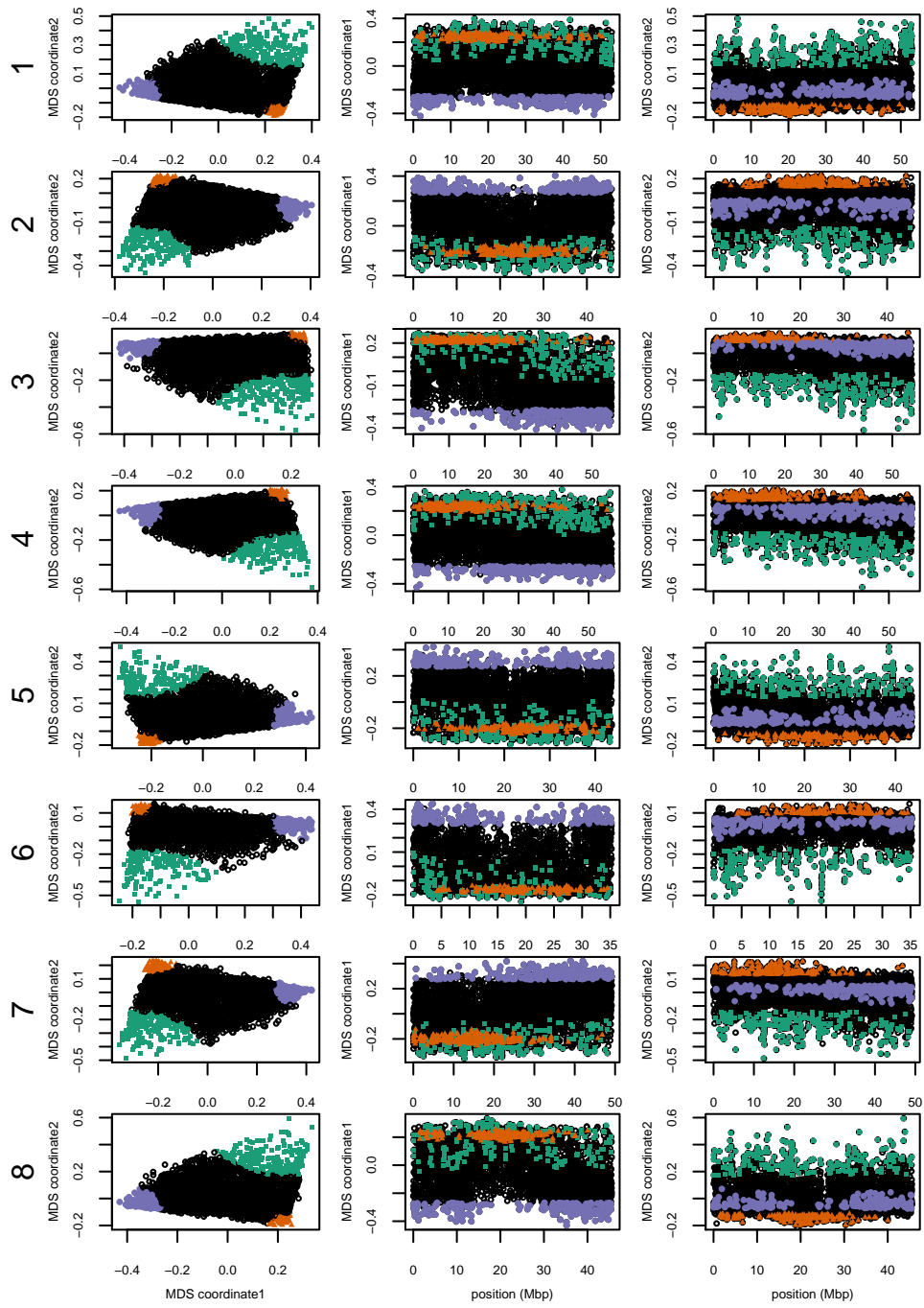


Figure 15: MDS plots for all 8 chromosomes in *Medicago truncatula*.

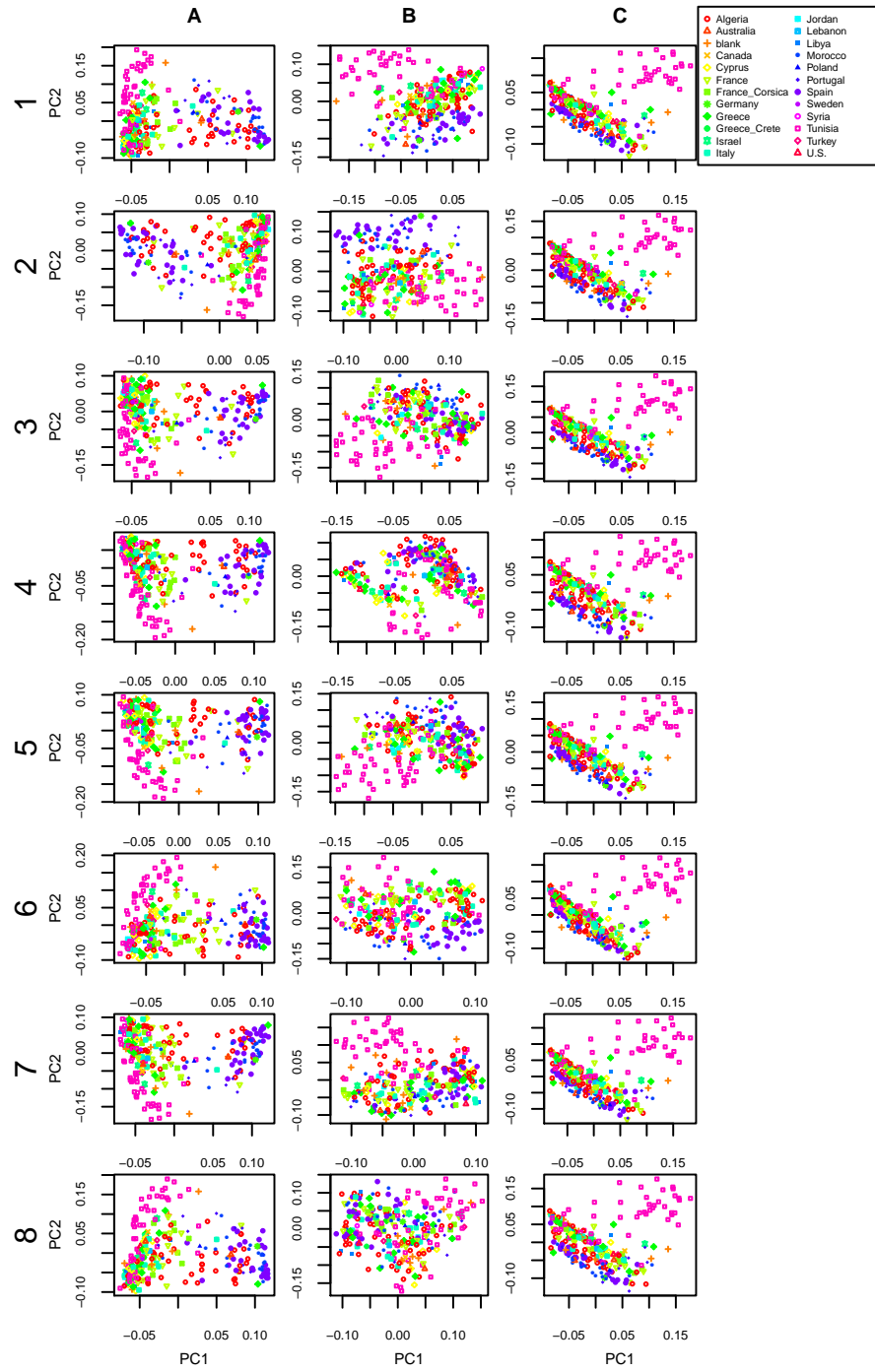


Figure 16: PCA plots for all 8 chromosomes' 3 peaks in *Medicago truncatula*. (A) green, (B) orange, and (C) purple.

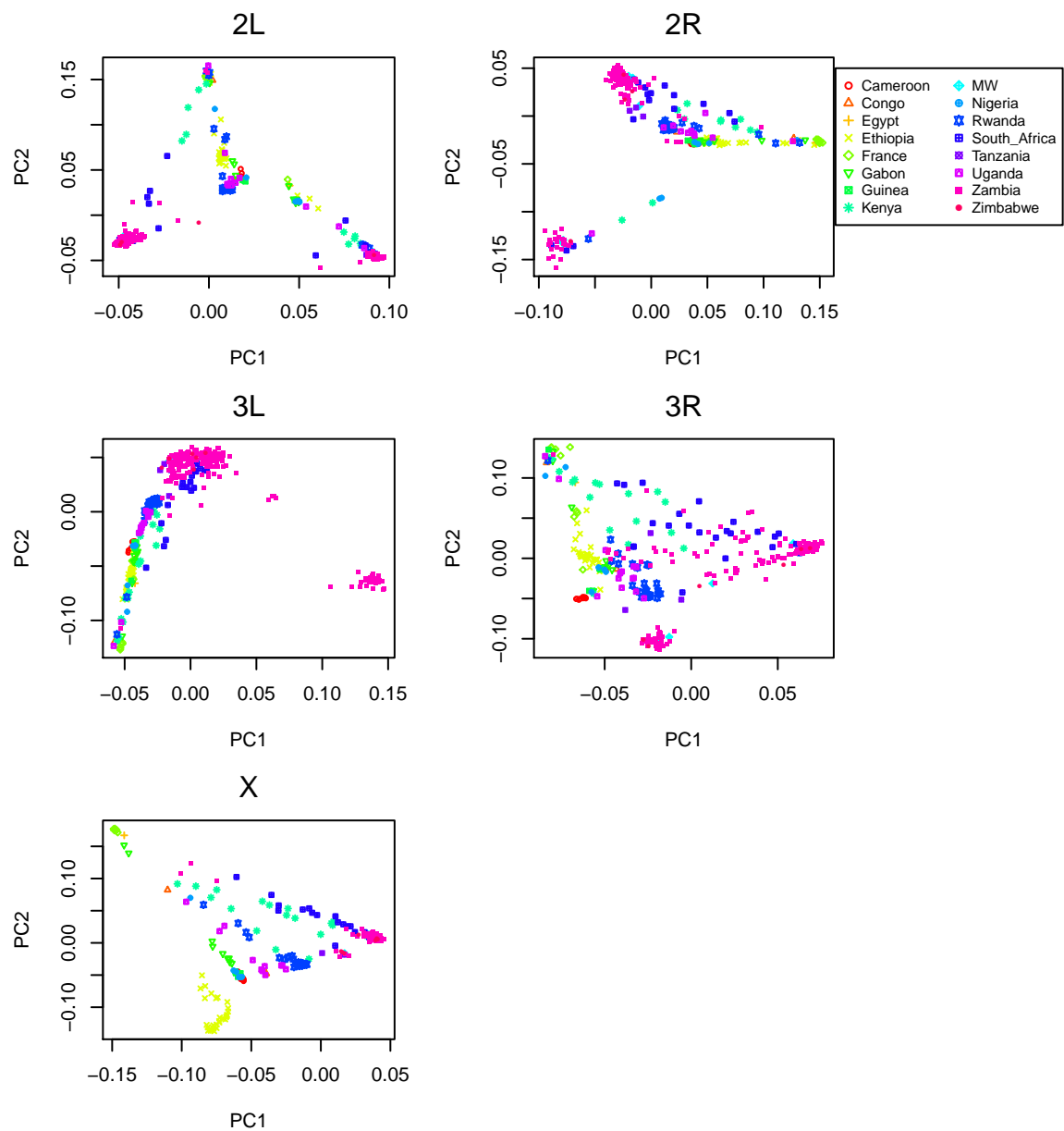


Figure 17: PCA plots for chromosome arms 2L, 2R, 3L, 3R and X in *Drosophila*.

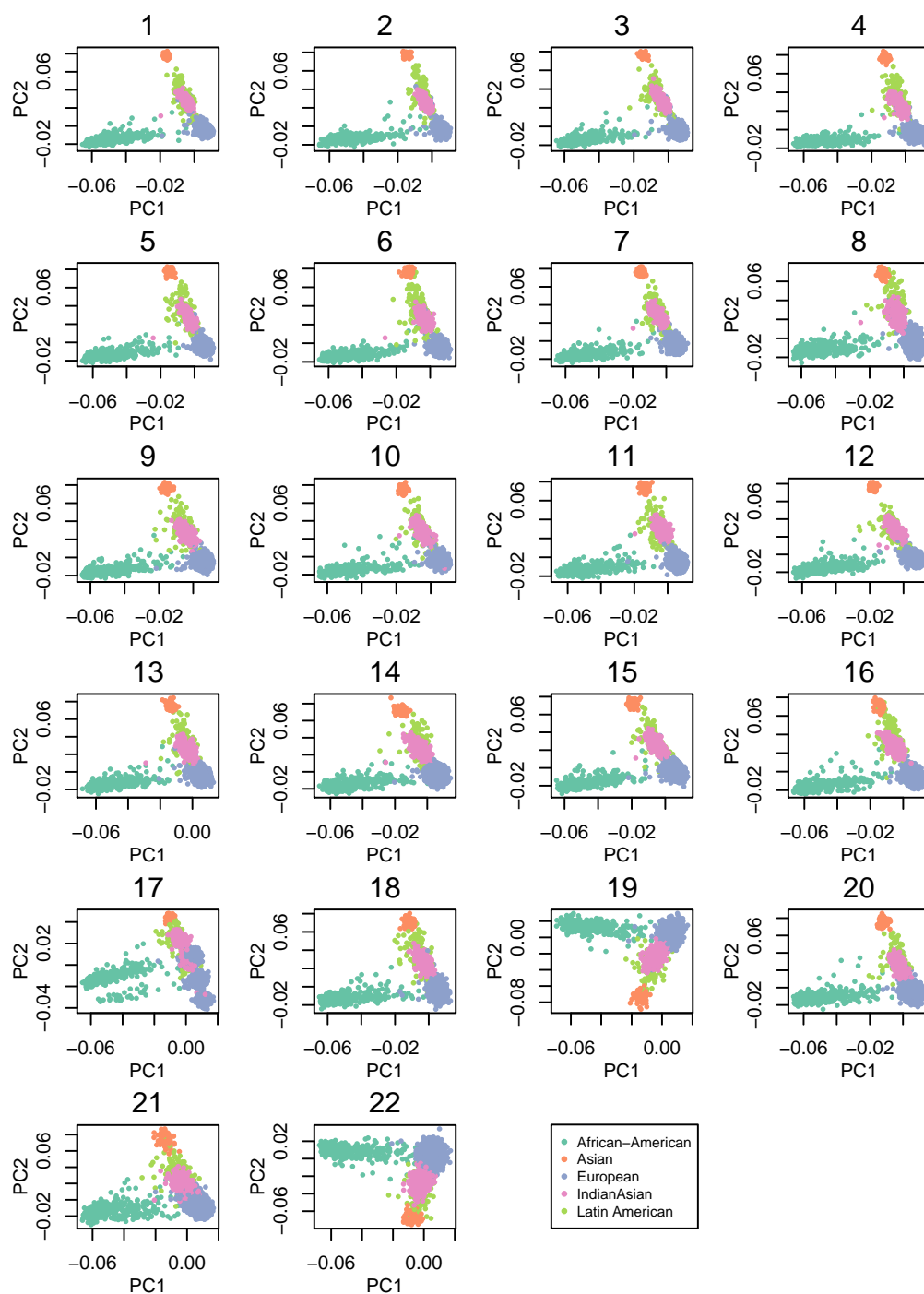


Figure 18: PCA plots for all 22 autosomes in human.

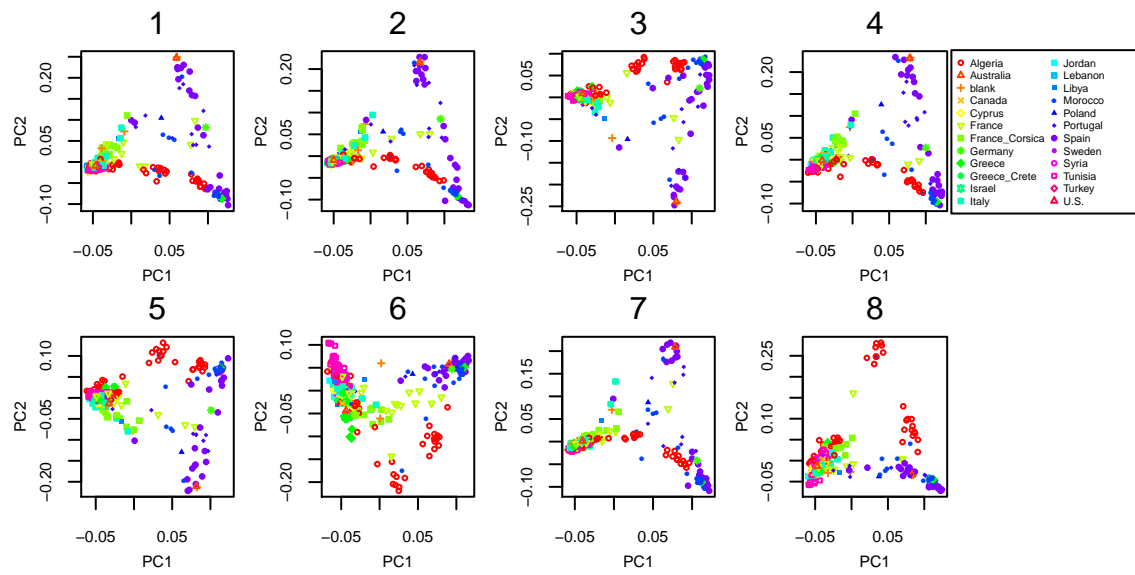


Figure 19: PCA plots for all 8 chromosomes in *Medicago truncatula*.