An Application of PC-AiR Method for Population Structure Inference in the Presence of Sample Relatedness

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Abstract

In this final project, we introduce the PC-AiR method [1] for robust inference of population structure when there exist related individuals. We describe the PC-AiR approach in detail and especially examine the shrinkage phenomenon when predicting the PC scores. To evaluate the performance and compare it with other methods, we apply it as well as competing methods in a simulation study and two real datasets.

Keywords: PCA, GWAS, Relatedness, Population structure

1. Introduction

Genome-wide association study (GWAS) is an approach to find genetic variations associated with a particular disease or trait via scanning markers across the genomes of many independent individuals [2]. In genome-wise association studies, it’s well known the unobserved population stratification might be a strong confounding variable. Traditional principle component analysis (PCA)-based methods have been successfully applied to correct for the population stratification [3, 4]. Specifically, principle component analysis is performed on the original feature matrix or the residual matrix after removing the effects of the observed variables. Similar to PCA-based methods, Multi-dimensional scaling (MDS) is another commonly used dimension reduction method to solve this problem. Alternatively, model-based methods for proportional ancestry estimation are also widely applied, such as such as STRUCTURE [5], FRAPPE [6], and ADMIXTURE [7].

In practice, genetic studies often include related individuals; however, most of existing population structure inference methods fail in the presence of relatedness. For PCA and MDS-based methods, the top ranked principle components (PCs) may reflect the family relatedness rather than population structure when applied to samples that include relatives [8]. The reason is the variation of related family numbers,
instead of the variation from the population structure, is detected and estimated from the high-dimensional feature matrix. When this happens, the top ranked PCs may be misleading. For model-based methods, they are also usually not able to distinguish between population groups and the clusters of relatives [9], since it is hard to split the two effects without additional information. In the family-based study designs with known pedigrees, SNP loadings calculated from performing principle component analysis on the pedigree founders may be used to obtain PC scores for genotyped offspring [10]. However, this method may fail when the relatedness information is unknown or misspecified. Therefore it can not be applied into most GWAS studies, where the genealogical information on the sample individuals is often incomplete or unavailable.

In this final report, we introduce the principle component analysis in related samples (PC-AiR) [1] method which is recently published. The PC-AiR method addressed the problem of population structure inference in the presence of related individuals. The performance and accuracy of the PC-AiR as well as several other competing methods are illustrated in simulation studies and real data analysis. In addition, we examine the shrinkage phenomenon when predicting the PC scores [11]. Without surprise, the PC-AiR method does not address the shrinkage problem and the predicted PC scores are biased toward to zero.

2. Review of the PC-AiR Method

2.1. An overview of PC-AiR

This is an overview of the PC-AiR method:

- \( N \): donated as the set of all simple individuals
- Partition \( N \) into two non-overlapping subsets, donated as \( U \) and \( R \)
- \( U \): subset of mutually unrelated individuals who are representative of the ancestries of all individuals in set \( N \)
- \( R \): “Related subset” of individuals who have at least one relative in \( U \)
- Two-step procedure:
  1. Performing PCA on individuals in \( U \)
  2. Predicting values along the components of variation for individuals in \( R \), based on genetic similarities with the individuals in \( U \).
2.2. Notation and Assumptions

Suppose the individuals in $N$ are sampled from population with ancestry derived from $K$ ancestral sub-populations. For SNP $s \in S$, donate $p_s = (p_1^s, ..., p_K^s)^T$ to be the vector of sub-population-specific allele frequencies, where $p_k^s$ is the allele frequency at SNP $s$ in sub-population $k \in \{1, ..., K\}$. Assume $p_k^s$ are random variables that are independent across $s$ but with possible dependence across the $k'$s. For the mean model of allele frequencies, the PC-AiR assumes $E[p_s] = p_s 1$; $Cov[p_s] = p_s (1-p_s) \Theta_K$ for each $s \in S$, where $\Theta_K$ is a $K \times K$ matrix. The matrix $\Theta_k$ is allowed to be general. A special case is the Balding-Nichols model \cite{12}, where $\Theta_k$ is a diagonal matrix with $(k,k)$-th element equal to $\theta_k \geq 0$, and $\theta_k$ is Wright’s standardized measure of variation, $F_{ST}$, for sub-population $k$. In the simulation studies, we use this model to generate population structure.

The PC-AiR allows for sample individuals to have admixed ancestry from the $K$ sub-populations, donate $a_i = (a_1^i, ..., a_K^i)^T$ to be the ancestry vector for individual $i \in N$, where $a_k^i$ is the proportion of ancestry across the autosomal chromosomes from sub-population $k$ for individual $i$, $a_k^i \geq 0$, $\sum a_k^i = 1$. In practice, $K, \Theta_K, p_s, p_s$ are unknown. The goal of PC-AiR is to obtain inference on ancestry, i.e. the $a_i$'s, for all the sample individuals $i \in N$ in the presence of known or unknown relatedness.

2.3. Relatedness Inference in Structured Populations

To distinguish the close relatives with similar ancestry from unrelated individuals, the PC-AiR uses kinship coefficients to measure genetic relatedness between all pairs $(i,j)$ of individuals in $N$. Recall the $\theta_{ij}$ is the probability that a random allele selected from $i$ and a random allele selected from $j$ at a locus are IBD. When the genealogy is known, the $\theta_{ij}$ can be theoretically derived. This may be refereed as pedigree-based kinship coefficients; when the genealogy is unknown or incomplete, the PC-AiR may use empirical estimates, the KING-robust estimator \cite{13} is recommended. It’s notable that the KING-robust is consistent estimator for pairs from the same sub-population but it is negatively biased for pairs of individuals that have different ancestries.

2.4. Measuring Ancestry Divergence

Identifying $U$ only on kinship coefficients can result in a subset which lacks sufficient diversity for population structure inference on the entire sample, as it may not be representative of the ancestries of all individuals. Therefore in additional to the measure of genetic relatedness, PC-AiR incorporates measures of ancestry divergence.
For \( i, j \in N \), let \( g_{is}, g_{js} \) be the numbers of copies of the reference allele that individuals \( i \) and \( j \) each have, at SNP \( s \in S_{ij} \subset S \), where \( i, j \) have non-missing data at \( S_{ij} \). Usually \( g_{is}, g_{js} \) take values of 0, 1, or 2. The estimator to measure the ancestry divergence between a pair of unrelated \( i, j \) is defined as:

\[
\hat{k}_{ij} = \frac{1}{2} \left( 1 - \frac{\sum_{s \in S_{ij}} (g_{is} - g_{js})^2}{\sum_{s \in S_{ij}} (1_{[g_{is}=1]} + 1_{[g_{js}=1]})} \right)
\]

where \( 1_{[g_{us}=1]} \) is an indicator for \( u \) being heterozygous at SNP \( s \). This is a generalized version of KING-robust. Recall \( i, j \) are assumed with admixed ancestry from \( K \) ancestral sub-populations. For unrelated individuals \( i, j \) from the same sub-population, \( \hat{k}_{ij} \to 0 \), as \( |S_{ij}| \to \infty \). When \( i, j \) have different ancestral backgrounds, \( \hat{k}_{ij} \) is negatively biased estimator of kinship, and the magnitude of this bias provides a useful measure of ancestry divergence between \( i, j \). PC-AiR uses the \( \hat{k}_{ij} \) to measure the ancestry divergence for all pairs of individuals \( i, j \in N \) who are NOT inferred to be related based on the kinship coefficient measures.

2.5. Identification of an Ancestry Representative Subset

The PC-AiR uses both the relatedness and ancestry divergence measures mentioned in section 2.3 and 2.4 to identify the subset \( U \). Specifically, a relatedness threshold \( \tau_\theta \) is predetermined, then \( i, j \) is said to be related if its kinship coefficient \( \hat{\theta}_{ij} > \tau_\theta \). For \( i \in N \), calculate \( \gamma_i = \sum_{j \neq i} \hat{\theta}_{ij} 1_{[\hat{\theta}_{ij}>\tau_\theta]} \) as a measure of the total kinship individual \( i \) has with inferred relatives in the sample. Similarly, predetermined divergence threshold \( \tau_k \) is set and unrelated \( i, j \) is said to be divergent if \( \hat{k}_{ij} < -\tau_k \), i.e. they have different ancestral backgrounds. For \( i \in N \), calculate \( \delta_i = \sum_{j \neq i} 1_{[\hat{\theta}_{ij}<\tau_\theta, \hat{k}_{ij}<-\tau_k]} \), the number of divergent ancestry pairs that individual \( i \) is a member of. Highest \( \delta_i \) values generally correspond to individuals with unique ancestry and/or individuals with an ancestry proportion close to 1 from one of the \( K \) sub-populations. In summary, to generate subset \( U \), the priority for inclusion in \( U \) is given to the individuals who is a member of the most divergent ancestry pairs (large \( \delta_i \)) and secondary priority is given to individuals share the most genetic information with their collection of relatives in \( N \) (large \( \gamma_i \)). A detailed algorithm of the PC-AiR method is displayed in Figure 1 and can also be founded in Appendix B of [1].
2.6. Genetic Similarity Matrix for PC-AiR

In traditional PCA-based methods, we perform PCA on standardized genotypes. For individual \(i\) and SNP \(s\), we construct

\[
z_{is} = \frac{g_{is} - 2\hat{p}_s}{\sqrt{2\hat{p}_s(1 - \hat{p}_s)}}
\]

(2)

where \(\hat{p}_s\) is typically an allele frequency estimate for SNP \(s\) calculated using all sample individuals. In PC-AiR method, the standardized genotypes are also used but the allele frequencies used for the standardization are calculated using only the unrelated individuals in \(U\), that means

\[
z_{is} = \frac{g_{is} - 2\hat{p}_u}{\sqrt{2\hat{p}_u(1 - \hat{p}_u)}}
\]

(3)

where \(\hat{p}_u = \frac{1}{|U|} \sum_{i \in U_s} g_{is}\), \(U_s\) is the subset of individuals in \(U\) who have non-missing genotype data at SNP \(s\). Quality control filtering of SNPs with poor quality is performed and finally we have filtered set \(S^*\) and construct

- \(Z\), an \(n \times |S^*|\) standardized genotype matrix for \(N\)
- \(Z_u\), an \(n_u \times |S^*|\) standardized genotype matrix for \(U\)
- \(Z_r\), an \(n_r \times |S^*|\) standardized genotype matrix for \(R\)

In the first step, we will construct the \(n_u \times n_u\) Genetic Similarity Matrix (GSM) using the individuals in \(U\):

\[
\hat{\Psi}_u = \frac{1}{|S^*|} Z_u Z_u^T
\]

(4)

GSM measures the average genetic similarity for individuals \(i, j\). Then a single value decomposition is performed on the GSM matrix

\[
\hat{\Psi}_u = V_u L_u V_u^{-1}
\]

(5)

Since individuals in \(U\) are mutually unrelated and have diverse ancestry, the top PCs are expected to be representative of ancestry. To predict the PCs for subset \(R\), a \(|S^*| \times n_u\) SNP weight matrix \(\hat{W}_u = Z_u^T V_u\) is considered, it measures the relative influence of each SNP on each of the \(n_u\) axes of variation in GSM \(\hat{\Psi}_u\). By \([14]\), \(n_u \times n_u\) matrix of PCs for \(U\) can be written as

\[
V_u = \hat{\Psi}_u V_u L_u^{-1} = \left(\frac{1}{|S^*|} Z_u Z_u^T\right) V_u L_u^{-1} = \frac{1}{|S^*|} Z_u W_u L_u^{-1}
\]

(6)
The $n_r \times n_u$ matrix of predicted PCs for $R$ is given as
\[
Q_r = \frac{1}{|S^*|} Z_i W_u L_u^{-1} \quad (7)
\]
The $d^{th}$ column vector of the matrix $Q_r$ corresponds to PC-AiR’s predicted coordinates along the $d^{th}$ axis of variation for the individuals in $R$. Therefore the combined $n \times n_u$ matrix of PCs for $U + R = N$ is obtained by combining $V_u$ and $Q_r$.

3. Shrinkage Phenomenon

In this section we discuss the shrinkage phenomenon of predicted PC scores of PC-AiR methods.

Predicted PC scores for a new sample are usually estimated in the “naive” fashion, which means the data vector of the new sample multiplied by the sample eigenvectors from the original PC analysis. Specifically, for the new individual with observation $x_{new}$, the predicted PC scores of this new observation on each of the PCs are defined as:
\[
U^T x_{new} \quad (8)
\]
where the matrix $U$ is the eigenvector matrix. When $p >> n$, the predicted PC scores tend to be biased and shrunken toward 0 [11].

By the simulation results displayed in Figure 2 and 3, we find that though the PC-AiR method works to identify the population structure, the predicted PC Scores using the proposed method are biased toward 0. In other words, PC-AiR does not address the shrinkage in PC scores prediction. In the PC-AiR method, the individuals selected into $U$ subset have large $\gamma_i$ and large $\delta_i$, which means this method tends to choose individuals with the highest ancestry proportions from each of the $K$ sub-populations for $U$. These individuals will be at the extremes of the $K - 1$ dimensional space spanned by the axes of variation representing the ancestries in $N$. This helps the PC-AiR method avoid part of the shrinkage in predicting the principal component scores. The detailed setting of Figure 2 can be founded in [11] and setting of Figure 3 will be explained in next section.

4. Simulation

We perform simulation studies in which both population and pedigree structure are simultaneously present in order to (1) assess the accuracy and robustness of the PC-AiR method for population structure inference in the presence of relatedness,
evaluate correction for population stratification with PC-AiR in genetic association studies with cryptic structure, and (3) compare the performance of PC-AiR to existing methods. We simulate a variety of population structure settings, including admixture and ancestry-related assortative mating, with differentiation between populations ranging from subtle to large. We evaluate population structure inference based on some relationship configurations, where each configuration corresponds to a specific setting of genealogical relationships among the sample individuals. In all simulation studies considered, pedigree information on the sample individuals is hidden and genetic relatedness is inferred from the genotype data for the PC-AiR method using the KING-robust kinship estimators.

We consider population structure settings where individuals have ancestry derived from two populations, and the allele frequencies at 5000 SNPs for each of these two populations are generated using the BaldingNichols model (Balding and Nichols, 1995). More precisely, for each SNP s, the allele frequency \( p_s \) in the ancestral population is drawn from a uniform distribution on \([0.1, 0.9]\), and the allele frequency in population \( k \in \{1, 2\} \) is drawn from a beta distribution with parameters \( p_s(1-\theta_k)/\theta_k \) and \((1-p_s)(1-\theta_k)/\theta_k \). In all simulations, we set \( \theta_1 \) and \( \theta_2 \) equal to a common \( F_{st} \) value. We consider \( F_{st} \) values of 0.005 and 0.01, 0.05 respectively, to generate allele frequencies from closely related and divergent populations.

For all three \( F_{st} \) values considered, we simulate a population structure which consist of individuals sampled from an admixed population formed from populations 1 and 2. From them, all unrelated individuals and pedigree founders have ancestry proportions \( a \) from population 1 and \((1-a)\) from population 2, with the parameter \( a \) for each individual drawn from a uniform distribution on \([0, 1]\). Offsprings which are considered as related samples are generated by picking the random pair of pedigrees within each group. In our setting, all ancestries have 20 samples for population 1,2 and mixture. With each group, we select 10 pairs of pedigrees to generate 20 offsprings, i.e. each pair produces 2 offsprings. To sample pedigree relationships within each group, we simulate genotypes for pedigree founders under HardyWeinberg equilibrium (HWE) and then drop alleles down the pedigree. Therefore, we have total 120 samples including all related and unrelated ones and SNP dimension \( p = 5000 \). Then we will apply PC-AiR method to infer the population structure for this dataset and then compared with some common method such as MDS and EIGENSOFT.

Figure 4, 5, 6 give the results of PC-AiR, EIGENSOFT and MDS, respectively. It shows that all the methods can have good performance for separating population groups. To assess the performance of three methods, we included the top principal components (axes of variation) from each method as predictors for the true simulated ancestry of the sample individuals in a linear regression model, and the proportion
Table 1: Proportion of ancestry explained ($R^2$) by different methods in simulation studies. $d$ denotes the number of axes of variation included as predictors in the linear regression model to determine the $R^2$ values. $d^*$ is the number of axes of variation that are required to achieve an $R^2$ of 0.90.

| $F_{st}$ | PC-AiR $d = 1$ | EIGENSOFT $d = 1$ | EIGENSOFT $d = 2$ | MDS $d = 1$ | MDS $d = 2$ | MDS $d^*$ |
|---------|----------------|------------------|------------------|-------------|-------------|-----------|
| 0.05    | 0.9917         | 0.9737           | 0.9738           | 0.9744      | 0.9744      | 1         |
| 0.01    | 0.9012         | 0.7365           | 0.7501           | 0.7437      | 0.7529      | 27        |
| 0.005   | 0.6796         | 0.2464           | 0.3868           | 0.2343      | 0.3592      | 36        |

of ancestry explained, as measured by $R^2$, was used to evaluate prediction accuracy. We also compared the efficiency of PC-AiR to EIGENSOFT by assessing the number of top axes of variation required to attain an $R^2$ of at least 0.90 for ancestry. From Table 1, PC-AiR has better performance than other two methods.

5. Real Example

In this section, we will apply PC-AiR methods into two datasets and evaluate its performance with other methods. Also, we can get some gene associations that are consistent with current literatures.

5.1. HapMap ASW and MXL Data

We analyzed high-density SNP genotype data from the combination of African American individuals in the southwestern USA (ASW) and Mexican Americans in Los Angeles, California (MXL) from HapMap 3 for population structure inference. We applied PC-AiR, EIGENSOFT, MDS to the 173 genotyped individuals. We want to classify these two groups and identify ancestry population and offsprings. Noticing that HapMap MXL and ASW samples have very different ancestral backgrounds. Most of the HapMap ASW ancestry is African, with a mean of 77.5% (SD = 8.4%). There is also a large European ancestry component, with a mean of 20.5% (SD = 7.9%); however, unlike the HapMap MXL, there is very little Native American ancestry in the HapMap ASW, with a mean of only 1.9% (SD = 3.5%). Since there are three predominant continental ancestries in the combined HapMap ASW and MXL samples, we expected that an optimal method would require two axes of variation to fully explain continental population structure. Figure 7, Figure 8, Figure 9 show the results of PC-AiR, EIGENSTRAT and MDS methods, respectively. It shows that for PC-AiR, it can correctly identify the related and unrelated samples and also have a great performance for classifying the population from ASW and MXL. For the other two methods, they also have good performance for inferring the
population structure. However, there exists some "outliers" in top of the figures. Actually, they are some related samples from MXL dataset.

5.2. IGSR and the 1000 Genomes Project

The International Genome Sample Resource (IGSR) was established to ensure the ongoing usability of data generated by the 1000 Genomes Project. The goal of the 1000 Genomes Project was to find most genetic variants with frequencies of at least 1% in the populations studied. The 1000 Genomes Project took advantage of developments in sequencing technology, which sharply reduced the cost of sequencing. It was the first project to sequence the genomes of a large number of people, to provide a comprehensive resource on human genetic variation. Data from the 1000 Genomes Project was quickly made available to the worldwide scientific community through freely accessible public databases. Our dataset contains samples $n = 1015$ from 14 different regions and SNP variables $p = 4967$. In each region, populations was compromised by families, i.e. it contains parents and their offsprings. Therefore, the dataset have many unrelated and related samples. We download the dataset from SNPedia [10] and apply PC-AiR methods to infer the data structures.

Figure 10 shows the results of PC-AiR for classifying population among 14 regions. It can separate some regions but still have many overlap between some populations such as Japanese, Han Chinese and Southern Han Chinese. These population are geographically close to each other, they should share more similar genotypes. When we roughly classify these 14 regions into 4 groups, i.e. "East Asia", "Africa", "America" and "Europe". It will have better performance for classifications as showed in Figure ???. It separates population from 14 regions into 4 main parts. Each part are geographically unrelated. Therefore, they should have less common genotypes.

6. Discussion

Genetic ancestry inference has been motivated by a variety of applications in population genetics, genetic association studies, and other genomic research areas. Advancements in array-based genotyping technologies have largely facilitated the investigation of genetic diversity at remarkably high levels of detail, and a variety of methods have been proposed for the identification of genetic ancestry differences among unrelated sample individuals using high-density genome-screen data. It is common, however, for genetic studies to have sample structure that is due to both population stratification and relatedness, and existing population structure inference
methods can fail in related samples. We use PC-AiR, a method for robust population structure inference in the presence of known or cryptic relatedness. PC-AiR applies a computationally efficient algorithm that uses pairwise measures of kinship and ancestry divergence from genome-screen data for the identification of a diverse subset of mutually unrelated individuals that is representative of the ancestries in the entire sample. Principal components that are representative of ancestry are obtained by performing PCA directly on genotype data from the mutually unrelated individuals selected for the ancestry representative subset, while coordinates along the axes of variation for the remaining individuals in the sample are predicted based on genetic similarities with the diverse subset. The PC-AiR method does not require the genealogy of the sampled individuals to be known, and it can be used across a variety of study designs, ranging from population-based studies where individuals are often assumed to be unrelated, to family-based studies with partially unknown or misspecified pedigrees.

Further study including reduce the shrinkage phenomenon which may introduce the weights between PC scores in unrelated samples for predicting the related groups and provide shrinkage population structure inference if given the reference population panels, external SNP loadings, or genealogical information on the sample individuals.

7. References

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PC-AiR Algorithm for Partitioning $\mathcal{N}$ into $\mathcal{U}$ and $\mathcal{R}$

1. Compute: $\gamma_i = \sum_{j \neq i} \hat{\phi}_{ij} 1_{[\hat{\phi}_{ij} > \tau_0]}$ and $\delta_i = \sum_{j \neq i} 1_{[\hat{\phi}_{ij} < \tau_0, \hat{\tau}_i < \tau_0]}$ for all $i \in \mathcal{N}$.

2. Initialize the two subsets to be $\mathcal{U} = \mathcal{N}$ and $\mathcal{R} = \emptyset$, where $\emptyset$ is the empty set.

3. Compute: $\eta_i = \begin{cases} \sum_{j \neq i} 1_{[\hat{\phi}_{ij} > \tau_0]} & \forall i \in \mathcal{U} \\ 0 & \forall i \in \mathcal{R} \end{cases}$.
   - If $\max_i(\eta_i) > 0$, continue to step (4). Otherwise, the algorithm has completed.

4. Identify $T_1 = \{i | \eta_i = \max_j(\eta_j)\}$, the subset of individuals in $\mathcal{U}$ with the most relatives in $\mathcal{U}$. If $|T_1| > 1$, where $|T_1|$ is the number of elements in $T_1$, go to step (5). Otherwise set $T^* = T_1$ and go to step (8).

5. Identify $T_2 = \{i | \delta_i = \min_j(\delta_j)\}$, the subset of individuals in $T_1$ that are members of the least divergent ancestry pairs. If $|T_2| > 1$, go to step (6). Otherwise set $T^* = T_2$ and go to step (8).

6. Identify $T_3 = \{i | \gamma_i = \min_j(\gamma_j)\}$, the subset of individuals in $T_2$ that have the minimum total kinship with their inferred relatives. If $|T_3| > 1$, go to step (7). Otherwise set $T^* = T_3$ and go to step (8).

7. Randomly select one element from $T_3$ and define this element to be the set $T^*$.

8. Define the sets: $\mathcal{U}^* = \mathcal{U} \setminus T^*$ and $\mathcal{R}^* = \mathcal{R} \cup T^*$.

9. Update $\mathcal{U} = \mathcal{U}^*$ and $\mathcal{R} = \mathcal{R}^*$ and return to step (3).

Figure 1:
Figure 2:
Figure 3:
Figure 4: Plot of first two PCs with PC-AiR method with total number of observation \( n = 120 \) and number of SNPs \( p = 5000 \).

Figure 5: Plot of first two PCs with EIGENSTRAT method with total number of observation \( n = 120 \) and number of SNPs \( p = 5000 \).
Figure 6: Plot of first two PCs with MDS method with total number of observation $n = 120$ and number of SNPs $p = 5000$. 
Figure 7: Plot of first two PCs with PC-AiR method of MXL and ASW dataset.
Figure 8: Plot of first two PCs with EIGENSTRAT method of MXL and ASW dataset.
Figure 9: Plot of first two PCs with MDS method of MXL and ASW dataset.
Figure 10: Plot of population structure from 14 regions using PC-AiR.
Figure 11: Plot of population structure from 4 continents using PC-AiR.